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A NON-GAS-PRODUCING STRAIN OF THE HOG-CHOLERA BACILLUS ISOLATED FROM AN OLD LABORATORY CULTURE.

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It is not uncommon to find minor variations among strains of the same species of bacteria, and in cultures that have been kept for several years on artificial media such changes are relatively frequent. It is seldom, however, that one finds a variation in the action on the more commonly used carbohydrates. When grown in carbohydrate media contained in the fermentation tube as recommended by Theobald Smith (1) for the determination of the action of bacteria on carbohydrates, the constancy of the action of a given species is remarkable. This method is especially valuable in the differentiation of the members of the colon-typhoid group of organisms, and a deviation from the usual action is of interest in its relation to classification and to the possible formation of new species. One of our stock cultures of the hog-cholera bacillus, an organism belonging to the paratyphoid group, has shown such a deviation in that it has lost the power to form gas from a number of carbohydrates, while acid formation remains. A review of the literature shows that non-gas-producing paratyphoid strains have been reported several times.

Dorset (2) describes an organism which he obtained from the spleen of a pig dying of acute hog-cholera. This organism was not agglutinated by the serum of a typhoid patient or by the dried serum of a guinea pig injected with this non-gas-producing organism.

Preisz (3), Bock (4), Grabert (5), and Bainbridge (6) have described cultures obtained from swine, which resembled the hog-cholera bacillus except that they failed to produce gas from dextrose.

Christiansen (7) isolated from nineteen calves non-gas-producing organisms which were agglutinated by an anti-Gaertner and an antiparacolon serum. The

latter serum protected to some extent mice injected with these organisms. After long cultivation on a variety of media he did not succeed in getting the organisms to form gas from dextrose.

Wagner (8) isolated from the blood of a woman *B. typhosus* and a non-gas-producing paratyphoid bacillus. 6 days later he isolated from the same patient a typical gas-producing paratyphoid. The last two organisms were agglutinated by an antiparatyphoid β serum and their carbohydrate reactions were the same, except that no gas was formed by the first organism.

Oette (9) and Ohno (10) have isolated from patients organisms that were agglutinated by paratyphoid β serum and that culturally corresponded to this group except that they failed to produce gas from dextrose and other carbohydrates.

It is not our purpose to review the many papers on bacterial mutations, for a review, with the literature on the subject, can be found in the work of Eisenberg (11). There are, however, a few papers that are directly related to the culture to be discussed.

Loewenthal and Seligmann (12) describe a non-gas-producing paratyphoid that they found in one of their stock cultures. This culture was isolated in 1908 in connection with the study of a meat poisoning epidemic and corresponded to paratyphoid β. For 3 years it was kept on slant agar, transfers being made every 6 to 8 weeks. In 1911 they began using it for diagnostic purposes and transfers were made two or three times a week. A year and a half later it was found that the culture no longer produced gas in dextrose or mannite media, while the original stock had retained this power. Passage through animals did not restore this lost property.

Hübener (13) found that paratyphoid bacilli grown at 46°C. lost the power to form gas from dextrose, but this power was quickly regained when the culture was again incubated at 37°C.

Penfold (14), growing paratyphoid organisms on agar plates containing sodium mono-chloracetate, obtained subcultures that failed to produce gas in dextrose and maltose media, while they retained the power to form gas from dulcite, mannite, and sorbite.

The culture on which we wish to report was received from Dr. Dinwiddie in 1899 and has been kept in stock under the name of Hog-cholera Ark. In 1901–02 Smith and Reagh (15) passed it through a series of thirteen rabbits. A bouillon culture from the heart's blood was used for each succeeding inoculation. Since that time this rabbit culture as well as the original stock has been kept on slant agar, transfers being made every 4 weeks. In 1914 the culture that had been passed through the rabbits was plated and to our surprise we found that transfers from over half the colonies studied failed to form gas in dextrose bouillon.

TABLE I.

Carbohydrates from Which Hog-Cholera Bacilli Form Gas.

Culture.	Results of titrations of fermented bouillon plus the following carbohydrates after 5 days at 37°C.											
	Dextrose.		Levulose.		Galactose.		Maltose.		Mannite.		Dulcite.	
	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.
Hog-cholera Ark., original.	Acid 4.2%	50% Gas.	Acid 4.1%	43% Gas.	Acid 3.5%	54% Gas.	Acid 4.1%	47% Gas.	Acid 2.2%	40% Gas.	Acid 0.5%	67% Gas.
Hog-cholera Ark., rabbit series, gas-forming.	Acid 4.0%	48% Gas.	Acid 4.0%	53% Gas.	Acid 3.5%	32% Gas.	Acid 3.2%	48% Gas.	Acid 1.7%	52% Gas.	Acid 3.0%	83% Gas.
Hog-cholera Ark., rabbit series, non-gas-forming.	**Acid 3.9%**	**No gas. Acid 3.8%**	**Acid 3.8%**	**No gas. Acid 3.1%**	**Acid 3.3%**	**No gas. Acid 3.0%**	**Acid 4.1%**	**No gas. Acid 4.2%**	**Acid 4.1%**	**No gas. Acid 3.9%**	**Acid 3.1%**	**No gas. Acid 3.0%**
Typhoid IX.	Acid 4.3%	Acid 4.1%	Acid 3.8%	Acid 3.9%	Acid 3.6%	Acid 3.3%						
Typhoid X.	Acid 4.4%	Acid 4.0%	Acid 4.2%	Acid 3.6%	Acid 4.0%	Acid 3.6%						

TABLE II.

Carbohydrates from Which Hog-Cholera Bacilli Do Not Form Gas.

Culture.	Results of titrations of fermented bouillon plus the following carbohydrates after 5 days at 37°C.									
	Lactose.		Saccharose.		Raffinose.		Dextrin.		Salicin.	
	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.	Bulb.	Branch.
Hog-cholera Ark., original.					Alkali.	Acid 0.7%	Alkali.	Large bubble of gas. Acid 1.6%*	Alkali.	Acid 0.4%
Hog-cholera Ark., rabbit series, gas-forming.	Acid 0.4%	Acid 1.2%	Neutral.	Acid 1.2%	Alkali.	Acid 0.7%	Alkali.	Small bubble of gas. Acid 1.3%*	Alkali.	Acid 0.5%
Hog-cholera Ark., rabbit series, non-gas-forming.	**Acid 0.5%**	**Acid 1.6%**	**Neutral.**	**Acid 1.3%**	**Alkali.**	**Acid 1.0%**	**Alkali.**	**Acid 1.6%***	**Alkali.**	**Acid 0.8%**
Typhoid IX.					Alkali.	Acid 0.7%	Alkali.	Acid 2.0%*	Alkali.	Acid 0.5%
Typhoid X.					Alkali.	Acid 1.0%	Alkali.	Acid 2.3%*	Alkali.	Acid 0.6%

* The original reaction of the bouillion was acid 0.8 per cent.

Cultural, Morphological, and Pathological Studies.

The non-gas-forming culture was compared with a gas-producing strain from the rabbit stock; with the original culture that had not been passed through the rabbits; and with two cultures of *Bacillus typhosus*. The non-gas-producing organisms from 24 hour agar and bouillon cultures cannot be differentiated morphologically from typhoid or the other strains of the hog-cholera bacillus, except that they are not quite as actively motile as the former. In 1894 Smith (16) pointed out, however, that in animals dying from an infection with the hog-cholera bacillus there was a marked morphological difference between the organisms found in tissues and those found in cultures. The non-gas-producing culture shows this same change in morphology under similar conditions, which, of course, differentiates it from the typhoid bacillus.

In bouillon it does not grow as readily as typhoid, but at the end of 5 days the turbidity of the two cultures is about the same. The carbohydrate reactions of these cultures are given in Tables I and II. It will be seen that except for the loss of power to produce gas the action on the carbohydrates is the same as that of the parent stock. It differs from the typhoid bacillus in that it fails to act on dextrin as readily as the latter, but this difference is one of degree only.

In Table III, which gives the results of titrations made on fermentation tubes containing milk, it will be seen that hog-cholera bacilli are differentiated from typhoid bacilli in that they form alkali more readily in the presence of oxygen. Here too the difference is one of degree only, for in 20 days one of the typhoid cultures formed as much alkali as the hog-cholera cultures (17).

The indol reaction of all the hog-cholera cultures tested was slight, while that of the typhoid cultures was negative.

It is interesting to note that together with the loss of power to produce gas from carbohydrates, this culture fails to decolorize neutral red dextrose agar, while the gas-forming strains decolorize this medium in 24 hours.

The agglutination tests given in Table IV sharply differentiate the non-gas-producing culture from the typhoid bacillus, but show no difference between the former and the other cultures of the hog-cholera bacillus.

TABLE III.

Growth of Hog-Cholera and Typhoid Bacilli in Fermentation Tube Containing Sterile Milk.

Culture.	After 10 days.			After 20 days.		
	Bulb.		Branch.	Bulb.		Branch.
	Condition.	Reaction.	Reaction.	Condition.	Reaction.	Reaction.
Hog-cholera Ark., original.	Clarified.*	Alkali. 0.6%	Acid 2.2%	Clarified.	Alkali 0.6%	Acid 2.2%
Hog-cholera Ark., rabbit series, gas-forming.	Clarified.	Alkali 0.6%	Acid 2.2%	Clarified.	Alkali 1.0%	Acid 2.2%
Hog-cholera Ark., rabbit series, non-gas-forming.	**Clarified.**	**Alkali 0.6%**	**Acid 2.3%**	**Clarified**	**Alkali 0.8%**	**Acid 2.2%**
Typhoid IX.	Not clarified.	Acid 0.6%	Acid 2.5%	Not clarified.	Alkali 0.3%	Acid 2.2%
Typhoid X.	Not clarified.	Acid 0.6%	Lost.	Clarified.	Alkali 0.7%	Acid 2.3%
Control tube.		Acid 1.5%	Acid 1.5%		Acid 1.5%	Acid 1.5%

* Partly translucent.

TABLE IV.

Agglutination Tests.

Culture.	Limit of agglutination.	
	Anti-typhoid serum.	Anti-hog-cholera bacillus serum.
Hog-cholera Ark., original	$\frac{1}{80}$	$\frac{1}{10,240}$
Hog-cholera Ark., rabbit series, gas-forming	$\frac{1}{80}$	$\frac{1}{10,240}$
Hog-cholera Ark., rabbit series, non-gas-forming	$\frac{1}{160}$	$\frac{1}{10,240}$
Typhoid IX	$\frac{1}{40,960}$	$\frac{1}{80}$
Typhoid X	$\frac{1}{40,960}$	$\frac{1}{80}$

The virulence of this non-gas-producing culture was compared with that of the gas-producing strain of the rabbit series and of the original stock by injecting known amounts of 24 hour bouillon cultures into rabbits of approximately equal weight. The results are summarized in Table V and show that the cultures passed through rabbits 15 years ago do not differ in their virulence, and are still markedly more virulent for the rabbit than the stock from which they originally came.

TABLE V.

Virulence Tests.

Culture. Hog cholera Ark.	Amount of 24 hr. bouillon culture injected into subcutis.	Weight of rabbit.	Died.
	cc.	*gm.*	*days*
Original	0.5	1,688	14
	0.1	1,406	24
Rabbit series, gas-forming	0.5	1,652	6+
	0.1	1,472	6−
Rabbit series, non-gas-forming	**0.5**	**1,642**	**8−**
	0.1	**1,535**	**6−**

The loss of power to produce gas from dextrose and other carbohydrates has persisted over a period of 18 months. The culture in the interval was kept on slant agar, and transfers were made monthly. Each transfer was incubated for from 24 to 48 hours and then placed in the refrigerator until the next culture was made. Rapid transfer through bouillon and transfers made directly from old agar slant growth to sugar tubes have failed to show a reversion to the original type of action. Passage through rabbits and mice has also failed to restore the power to produce gas.

DISCUSSION.

At first it was thought that the long cultivation on an artificial medium might have produced this change, but an examination of the other hog-cholera stocks showed that all the subcultures examined from colonies on agar plates produced gas in dextrose bouillon. One of these stocks had been in the laboratory for 30, another for 18 years, while three had been isolated more recently.

As the parent stock and the culture in which this new form occurred differed in that the latter had been passed through a series of rabbits, it seemed logical to assume that the animal transfers had been in some way responsible for the change. If this were the case, one should be able to produce a similar change in another strain. This was attempted by passing a recently isolated strain of the hog-cholera bacillus through a series of eleven rabbits, the inoculations being made by using a suspension of the crushed spleen of the animal dying of the disease to infect the next rabbit. The culture showed a marked increase in virulence, but no change in the gas production could be detected. From the last three rabbits in the series agar plates were made from the suspension of the crushed spleen and transfers made to dextrose Durham tubes from approximately fifty colonies in each case. All these tubes showed gas production.

Several environmental changes were tried in order to determine whether they would influence gas production. Transfers through twenty tubes of plain bouillon and bouillon containing 5 per cent of sodium chloride failed to influence gas production. Transfers through bouillon containing anti-hog-cholera bacillus serum were also without effect. Cultivation for $3\frac{1}{2}$ months in Dunham's peptone solution failed to yield cultures that differed from the original culture in their action on dextrose.

The negative results of attempts to influence gas production by means of environmental changes show that we are dealing with a highly stable property. The failure to influence it by the animal passages may mean that we selected a more resistant strain than the Hog-cholera Ark., or it may mean that the rabbit passages had nothing to do with the change that occurred in the latter culture. The possibility of this new form being a contamination can be ruled

out, for we have not had such a culture among our laboratory stocks; and the finding of paratyphoid bacilli in spontaneous diseases of rabbits has not to my knowledge been reported.

The loss or perhaps gain of cultural characters in a given strain is very different from the transformation of one species of bacteria into another, as has been recently reported by M'Gowan. In association with Wang (18), M'Gowan reports the transformation of *B. avisepticus* (fowl-cholera bacillus) by passing it through a series of fourteen guinea pigs, into a motile organism that forms acid and gas from a number of carbohydrates including lactose and saccharose. The serum of an animal immune to this motile organism did not agglutinate the original culture from which it is supposed to have come. In spite of the fact that this motile organism resembles *B. coli* more than any other, M'Gowan makes certain assumptions based on this transformation and the fact that in his hands members of the septicemia hemorrhagica group have shown marked variability in their cultural reactions. In discussing swine fever (19) he assumes that *B. suipestifer* (hog-cholera bacillus) is a variant of *B. suisepticus*, and in his paper on fowl-cholera (20) he assumes that *B. pullorum* and the fowl typhoid bacillus are variants of *B. avisepticus*.

These assumptions might explain many puzzling questions, but the facts on which they are based do not agree with our experience. Using the fermentation tube, as recommended by Smith (1) in determining the sugar reactions, members of the paratyphoid and septicemia hemorrhagica groups have shown marked stability in their cultural characters. There are essential differences between the cultural reactions of these two groups, the type of disease they produce when injected into animals is entirely different, and they have shown no cross immune reactions. Such transformations as M'Gowan reports must therefore be looked upon with doubt until we have more definite proof that they occur.

SUMMARY.

In a stock culture of the hog-cholera bacillus, which was passed through a series of rabbits 14 years ago, an organism was found that differs from the original culture in that it fails to form gas from the carbohydrates that are usually attacked by this organism, while acid formation persists. This new strain is agglutinated by an anti-hog-cholera bacillus serum and produces in rabbits and mice a disease

similar to that caused by the typical cultures. The failure to form gas has persisted over a period of 18 months and all attempts to cause the strain to revert to the original condition have failed. It resembles in many respects *Bacillus typhosus* and it may be that some of the so called typhoid cultures that are not agglutinated by antityphoid serum are non-gas-producing paratyphoids. Attempts to produce a similar change in a more recently isolated culture of the hog-cholera bacillus by means of animal passages and changes in the environment have been negative.

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THE EFFECTS OF EXPOSURE TO COLD UPON EXPERIMENTAL INFECTION OF THE RESPIRATORY TRACT.*

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Exposure to cold has long been considered an important factor in the incidence of many respiratory diseases. Even with our increasing knowledge of the predominating part of bacterial infection, the belief is still general among clinicians that chilling of the body exerts a predisposing influence of considerable importance.

In reviewing the general subject of the effect of atmospheric conditions upon bodily health and efficiency, the New York State Ventilation Commission was impressed with the comparative lack of accurate data concerning the physiological effect of low temperatures, the majority of recent observers having interested themselves mainly with the study of various degrees of heat.

Experimental studies of the influence of cold as well as of heat have therefore formed a part of the work of the Commission for the past 2 years. The experiments here reported constitute a portion of these studies.

EXPERIMENTAL.

The organism chosen for inoculation was *Bacillus bovisepticus*, or snuffles bacillus, which belongs to the hemorrhagic septicemia group and is pathogenic for the rabbit. It is a small, round bacillus with a marked tendency to bipolar staining, particularly in blood films made from animals dying from infection caused by it. The disease produced under natural conditions is generally a true septicemia, but in many cases is localized in the upper respiratory passages,

* Conducted under the auspices of the New York State Ventilation Commission.

when it is commonly known as snuffles. This condition is characterized by a typical discharge of yellowish mucus from the nostrils and may terminate in recovery or in a fatal pneumonia with or without general septicemia.

It will thus be seen that the conditions produced in the rabbit by this bacillus are similar to some of the respiratory infections in man, such as pneumonia and influenza. The organism was selected for use in our work because of this fact and because of the relative difficulties which have attended previous experiments with acute respiratory infections, particularly those caused by the pneumococcus.

Experiment 1.—This experiment was conducted during the late winter months of 1915 and 1916. Thirty-seven experimental rabbits were used in thirteen separate series of from two to four animals, each with an equal number of controls.

The experimental rabbits were kept in an incubator at warm temperatures for periods varying from 1 day to 1 week. In eleven series this temperature was between 85–90° F., and in two it varied from 70–76° F. and 63–78° F., respectively, being, however, at the higher level most of the time. The animals were then inoculated by spraying the nasal mucous membrane or the throat with a small amount of the live culture of *Bacillus bovisepticus*, emulsified in salt solution. After inoculation they were immediately chilled by exposing them to the outside weather for periods varying from ½ to 3 hours and at a temperature of 20–56° F. In the majority of the experiments the fur of the animals was wet with water at about body temperature, to facilitate chilling upon exposure. The details of temperature, time of exposure, method of inoculation, etc., are shown in the table under each experiment.

The control animals, also thirty-seven in number, were kept constantly at a temperature of 65–70° F. and were inoculated at the same time and in the same manner as the experimental animals of the corresponding series. None of these were wet.

Results.—Of the experimental rabbits five died of pneumonia, and one of general septicemia, while nine were made ill, but recovered. Of the controls three died and six others were infected, but recovered. In every case when death resulted, the animal was autopsied, the character of the lesions noted, and the specific organism isolated from the heart's blood.

It will thus be seen that of the experimental animals fifteen, or 40 per cent, reacted, and only nine, or 24 per cent, of the controls. It is also worthy of note that this difference in reaction between the experimental and the control groups was harmoniously distributed

throughout the entire thirteen series of experiments. In only two series (Nos. 11 and 12), did the number of reactions in the control group exceed that of the experimental animals. The details of this experiment are shown in Table I.

It seemed desirable to note the effect of reversing the conditions; that is, to determine the result of a change from a cold to a hot temperature. It was possible to conduct only two series in this experiment.

Experiment 2.—Four animals were kept at out of door temperature for 2 days, and after inoculation as in the previous experiment were placed in an incubator at 78–80°F. for from 6 to 12 days. A similar number of controls, inoculated in the same way, were kept constantly at room temperature (65–70° F.).

Results.—Of the experimental animals all reacted, of which one died. Of the controls only two reacted, one of which also died. A detailed description is given in Table II.

From the results obtained in the two foregoing experiments it seemed desirable to attempt one further experiment in which three modifications of the experimental conditions were considered together. (1) A change from outside temperature (59° F.) to incubator temperature (80° F.), (Series 16). (2) A change from room temperature (65–70° F.) to incubator temperature (80° F.), then down to a low temperature (50–60° F.), and from that back to room temperature (65–70° F.), (Series 17). (3) A modification of the conditions of Experiment 1; namely, chilling from a high temperature (80° F.) to a low one (57° F.), and then kept at 65-70° F. (Series 18).

Experiment 3.—All three series of this experiment were carried on simultaneously with two animals in each series, and with one set of two control animals which were kept continuously at room temperature (65–70° F.).

Results.—In Series 16, subjected to a change from low to high temperature, both animals died of pneumonia. In Series 17, subjected to several changes from room temperature, to high, then to low, and back to room temperature, both animals reacted, of which one died of pneumonia. In Series 18, subjected to a change from high to low temperature, only one animal reacted slightly with snuffles. Neither of the two controls reacted. Table III shows these results in detail.

TABLE I.

Series.	Heated and exposed.			Controls, kept at room temperature (65–70°F.).		
	No. of rabbits.	Reactions.		No. of rabbits.	Reactions.	
		Died.	Sick.		Died.	Sick.
1	4 24 hrs. at 87°F.; chilled to 45°F. for 1 hr. 2, fur wet; 2, fur dry. 2, nose; 2, throat.	2 Pneumonia. 1, fur wet; 1, fur dry. Death in 48 hrs.	1 General reaction; could hardly move; no appetite. Recovered.	4	0	1 General reaction. Recovered.
2	2 1 wk. at 85–89°F.; chilled to 40°F. for 1½ hrs. Fur dry. 1, nose; 1, throat.	0	1 Snuffles. Recovered.	2	0	0
3	2 1 wk. at 85–89°F.; chilled to 38°F. for 3 hrs. Fur dry. 1, nose; 1, throat.	0	1 Snuffles. Recovered.	2	1 Pneumonia. Death in 1 wk.	0
4	4 1 wk. at 85–90°F.; chilled to 38°F. for 1½ hrs. Fur dry. 2, nose; 2, throat.	0	0	4	0	0
5	4 1 wk. at 85–90°F.; chilled to 56°F. for 1 hr. Fur dry. 2, nose; 2, throat.	0	0	4	0	0
6	4 1 wk. at 85–90°F.; chilled to 20°F. for ½ hr. Fur dry. 2, nose; 2, throat.	1 Snuffles; septicemia. Death in 10 days.	0	4	0	0

7	4 1 wk. at 85–89°F.; chilled to 45°F. for 2 hrs. Fur dry. 2, nose; 2, throat.	0	1 Snuffles. Recovered.	4	0	1 Snuffles. Recovered.
8	3 1 wk. at 85–90°F.; chilled to 40°F. Fur wet.	0	2 Snuffles. Recovered.	3	0	0
9	2 3 days at 70–76°F.; chilled to 25°F. for ½ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 2 days. Inoculated in throat.	1 Snuffles. Inoculated in nose. Recovered.	2	2	0
10	2 3 days at 85–93°F.; chilled to 52°F. for ½ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 11 days. Inoculated in nose.	0	2	0	0
11	2 24 hrs. at 82–87°F.; chilled to 27°F. for ½ hr. Fur wet. 1, nose; 1, throat.	0	0	2	0	1 Snuffles. Recovered. Inoculated in nose.
12	2 24 hrs. at 85–90°F.; chilled to 31°F. for ½ hr. Fur wet. 1, nose; 1, throat.	0	1 Snuffles. Severe reaction. Recovered. Inoculated in nose.	2	0	2 Snuffles. Mild reaction. Recovered.
13	2 4 days at 63–78°F.; chilled to 32°F. for ½ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 3 days. Inoculated in throat.	1 Snuffles. Recovered. Inoculated in nose.	2	0	1 Snuffles. Recovered. Inoculated in nose.
Total	37	6	9	37	3	6

TABLE II.

Series.	Kept cold in the open, then heated in incubator.			Controls, kept at room temperature (65–70°F.).		
	No. of rabbits.	Reactions.		No. of rabbits.	Reactions.	
		Died.	Sick.		Died.	Sick.
14	2 2 days at 41°F.; 12 days at 80°F. 1, nose; 1, throat.	0	2 Snuffles. 1, inoculated in nose, reacted in 4 days; 1, inoculated in throat, reacted in 6 days.	2	0	1 Reacted in 12 days; Inoculated in nose.
15	2 2 days at 35–40°F.; 6 days at 78°F.	1 Pneumonia. Inoculated in throat.	1 Snuffles. Inoculated in nose.	2	1 Pneumonia. Inoculated in throat.	0
Total	4	1	3	4	1	1

TABLE III.

Experimental.									Controls, kept at room temperature (65–70° F.).		
Series 16.			Series 17.			Series 18.					
48 hrs. at 59° F.; inoculated and kept at 80° F. 1, nose; 1, throat.			48 hrs. at 65–70° F.; inoculated; 24 hrs. at 80° F.; 4 days in open (50–60° F.); then at room temperature (65–70° F.).			2 days' incubation (80° F); inoculated; fur wet; 20 min. at 57° F.; then kept at 65–70° F.					
No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.
2	2 Pneumonia.	0	2	1 Pneumonia. Death in 6 days. Inoculated in nose.	1 Mild attack of snuffles in 7 days. Inoculated in throat.	2	0	1 Snuffles in 3 days. Inoculated in throat.	2	0	0

DISCUSSION.

Other experimental studies of respiratory disease have been numerous, but few of these deal directly with the associated problem of the effect of chill with which we are at present concerned.

Lipari (1) in 1888 reports the results of intratracheal injections of pneumonic sputum in guinea pigs and rabbits which, after exercise by running, were chilled either by cold baths at 3°C. or by the application of ether to the skin after the animals had been shaved. Of eight chilled animals six died, while only two of twelve controls were affected.

Lode (2) in 1897 shaved guinea pigs over one-half to two-thirds of the body, exposed them to heat (37°C.) for half an hour, and then chilled them in draughts at open windows. Using Buchner's apparatus he exposed them to the spray of cultures of Friedländer's bacillus. Of eleven chilled animals seven died, while only three of eleven controls succumbed. In a similar inhalation experiment with tubercle bacilli, of five chilled animals all died, but only two of four controls. He also used Friedländer's bacillus, cholera bacillus, and *Staphylococcus aureus* subcutaneously in his series, as well as anthrax with a few chickens and rats. Altogether, including both inhalation and subcutaneous methods, he reports that forty-six of fifty-four chilled animals (85 per cent) died, and only nine of forty-five controls (20 per cent).

Pasteur's (3) experiments upon chickens made susceptible to anthrax by partial immersion in cold water are well known, but these as well as others not dealing with direct respiratory infection need not be considered here.

Chodounský (4), who has covered the whole field of the relation between exposure to cold and disease perhaps more extensively than any writer, comes to the conclusion that such exposure plays no part in determining the incidence of any of the various so called cold diseases. As a part of his contention he offers considerable experimental evidence concerning respiratory infections.

The experimental animals in Chodounský's studies were chilled by exposure to ice baths and cold draughts until their rectal temperature was lowered from 1–7°C. He employed several kinds of animals and several different pathogenic bacteria.

Virulent pneumococcus cultures by inhalation were used with one rabbit and one guinea pig with similar animals for controls. The rabbit was infected and died, but the guinea pig as well as the two controls survived.

Intravenous injections of pneumococci were also employed with practically identical results in the chilled and control animals.

Virulent cultures of Friedländer's bacillus were used by intratracheal injections in dogs and by inhalation with Buchner's apparatus in guinea pigs. Six dogs and two guinea pigs with a similar number of controls were employed. Of the dogs two experimental animals and three controls were infected; of the guinea pigs one each of the experimental animals and controls.

Chodounský also used cultures of Friedländer's bacillus attenuated by heat to determine whether these cultures might infect chilled animals. Intratracheal injections in four experimental dogs and four controls failed to infect any. Later, one of the controls was injected with a virulent culture and promptly succumbed. Chodounský also experimented with intravenous injections of pneumococcus in guinea pigs, with intraperitoneal injections of Friedländer's bacillus in rats, and with subcutaneous injections of diphtheria bacilli either alone or in combination with streptococcus, and in all these found no difference in reaction between the chilled and control animals.

More interesting and perhaps even more instructive are the results of Chodounský's self-experimentation. He describes himself as a man 57 years of age, subject to catarrh, frequent bronchitis, and lumbago. He reports in detail the results of twenty-seven separate experiments upon the effect upon himself of severe exposure to cold. After hot baths, after ice cold baths, and even when suffering from an acute coryza, he exposed himself naked to cold air and draughts at temperatures from 3–12°C. for periods of time varying from 20 minutes to 1 hour. Such exposure often caused severe shaking chills, but no permanent effect of any kind and no symptoms of any of the pathological conditions usually ascribed to exposure to cold.

Recently Kline and Winternitz (5), in the course of their studies of experimental pneumonia in rabbits, found that in the case of animals which were ex-

posed to cold (4°C.), after the ingestion of alcohol every day for periods varying from 1 to 9 days, four out of seven died, two of which showed intense laryngitis, tracheitis, and bronchitis, and one a bronchopneumonia. One other animal was killed at the end of 3 days and showed patchy congestion in the lungs. In the two remaining animals cultures of pneumococcus were injected intratracheally and death occurred in both, with lesions in the lungs and upper air passages. Pneumonia rarely occurred after such injections in animals not exposed to the effects of cold and of alcohol.

The weight of the experimental evidence thus reported appears to be against Chodounský's contention that exposure to cold has no influence upon respiratory disease, and with this evidence the results of our experiments are in accord.

The method of action of these secondary factors in infection is open to discussion. The lowering of general or specific resistance to infection may be considered. It has been shown by a number of observers including ourselves (6) that the production of such antibodies as hemolysins and agglutinins is affected by atmospheric temperature, especially, however, by heat. On the other hand, changes in the local resistance of the mucous membranes of the upper air passages may be more important. That definite circulatory changes do occur in the upper air passages as a result of varying atmospheric temperature has been observed in animals and also in clinical experiments upon human subjects by one of us (7) in collaboration with Cocks, which corroborated similar observations by Hill and Muecke (8). In these experiments the results appeared to be due to the direct action of air upon the mucous membranes and not to a reflex from the surface of the body. The possible concern of vagus control in this action, as suggested by Kline and Winternitz (5), is worthy of serious consideration. The fact that infection of the normal respiratory tract is experimentally difficult tends to emphasize the probable importance of the local changes in the mucous membranes favoring the entrance of bacteria as the main predisposing factor in the problem under consideration.

CONCLUSIONS.

1. Respiratory infection of rabbits with *Bacillus bovisepticus* (snuffles) is favored by chilling the animals after they have been accustomed to heat.

2. The character of this disease, which occurs frequently in rabbits under natural conditions, makes the application of the experimental results to similar respiratory conditions in man less open to objection than in similar experiments with other infections.

3. The weight of experimental evidence, including our own, does not justify the elimination of exposure to cold as a possible though secondary factor in the incidence of acute respiratory disease.

4. From the limited data of our last two experiments it is suggested that any marked change of temperature predisposes rabbits to this infection, the severity of which varies with the amount of change, and that a change from low to high temperature has an even more marked effect than that from high to low.

We wish to acknowledge our obligation to Mr. I. Weinstein for valuable assistance in conducting the details of the animal work in some of the experiments.

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STUDIES ON HETEROGENEOUS HEMOLYTIC SERA.

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In 1911 Forssman (1) refuted the theory of the specificity of antibodies which had been generally admitted since the discovery of bacteriological antibodies by Pfeiffer and of hemolysins by Bordet. Forssman demonstrated that rabbits immunized with organs of the guinea pig produced a hemolysin against sheep red blood corpuscles. The hemolytic serum thus obtained possessed greater hemolytic power for sheep red corpuscles than for those of the guinea pig. The minimum hemolytic dose for sheep red corpuscles was 0.001 cc., and for those of the guinea pig, 0.03 cc. It was afterwards found that the antigen-producing sheep hemolysin was found in other animals as well as in the guinea pig, *i.e.*, horse, cat, dog, mouse, hen, pigeon, turtle, toad (*Bufo*), carp, tench, pike, and eel, and in the cells of some bacteria (*Bacillus paratyphosus B* and Gärtner's bacillus). Forssman's finding that the antigen-producing heterogeneous sheep hemolysin is present in the organs of animals has been confirmed by Orudschiew (2) in serum, and by Doerr and Pick (3) in urine, into which it passes from the cells of the organs. According to Doerr and Pick (4), it is not found in the red corpuscles of these animals. Orudschiew, who first studied the problem, came to this conclusion because after immunizing rabbits with guinea pig red corpuscles he obtained serum containing hemolysin for guinea pig red corpuscles but not for sheep corpuscles. Doerr and Pick (5) state that an inverse relation exists, as a rule, between the organs of the animal and the red corpuscles. When the organs contain the antigen, the red corpuscles do not (horse, guinea pig, dog, mouse[1]), and *vice versa* (sheep). Morgenroth (6) held the same view. Friedberger and Schiff (7) state that one of three rabbits immunized with human red corpuscles produced a serum that dissolved sheep red corpuscles in a dose of 0.01 cc.;[2] but apparently Friedberger did not attach much value to this one positive experiment, because in his later works (8, 9) human red corpuscles are not mentioned as containing heterogeneous antigens.

[1] In another work Doerr and Pick (4) added the hen and the turtle to this list.

[2] The other two rabbits produced a serum of which the minimum dose did not exceed that of a normal animal; moreover, the authors do not cite protocols, and one cannot see whether the hemolysis in the only positive experiment (at 0.01 cc.) was complete or partial.

My experiments show that the connective tissue of animals (fat of guinea pigs) possesses an antigen-producing hemolysin against sheep red corpuscles. This fact, in connection with the works of Doerr and Pick (5) demonstrating that the heterogeneous sheep antigen found in animal organs was a nucleoprotein, made me believe that the cells not deprived of a nucleus, for example the red corpuscles with a nucleus derived from the same mesoderm as the connective tissue, should also contain heterogeneous sheep antigen when the organs of the same animal possess it. The experiments confirmed this hypothesis. The experiments were made with red corpuscles of the hen, whose organs, as shown by Doerr and Pick, contain a heterogeneous antigen-producing hemolysin against sheep red corpuscles.

EXPERIMENTAL.

Hen red corpuscles were washed three times in physiological salt solution. To the sediment left after centrifugation was added an equal part of salt solution, and the mixture was injected into rabbits. The serum was inactivated at 56° C. for ½ hour. Its hemolytic power was titrated in the presence of 0.1 cc. of guinea pig complement, which by itself is unable to cause hemolysis, and of 1 cc. of a 5 per cent suspension of the sediment of the sheep red blood corpuscles, in a total volume of 3 cc. The tubes were kept at 37° C., and the result of the experiment was read after 1 hour.

Rabbit 1.—Weight 1,625 gm. Jan. 8, 1.5 cc.[3] of hen red corpuscles injected into the ear vein. Jan. 15, 1.2 cc. injected. The hemolytic power of the serum tested on Jan. 22 and 26 was identical. 0.01 cc. produced complete hemolysis of sheep corpuscles; 0.005 cc., almost complete hemolysis.

Rabbit 2.—Weight 1,285 gm. Jan. 24, 2.0 cc. of hen red corpuscles injected into the ear vein. Jan. 31, 5 cc. injected into the abdomen. Hemolytic power: Feb. 8, 0.001 cc., complete hemolysis of sheep corpuscles; 0.0008 cc., almost complete hemolysis.

Rabbit 3.—Weight 1,775 gm. Jan. 24, 3.0 cc. of hen red corpuscles injected into the ear vein. Jan. 31, 2.5 cc. injected in two doses, first 0.25 and after ½ hour, 2.25 cc. Hemolytic power: Feb. 8, 0.002 cc., complete hemolysis of sheep corpuscles; 0.0016 cc., incomplete hemolysis.

Rabbit 4.—Weight 1,370 gm. Jan. 24, 2.0 cc. of hen red corpuscles injected into the ear vein. Jan. 31, 2.5 cc., in two doses. Hemolytic power: Feb. 8, 0.00125 cc., complete hemolysis of sheep corpuscles; 0.0011 cc., incomplete hemolysis.

Rabbit 5.—Weight 1,220 gm. Feb. 24, 2.5 cc. of hen red corpuscles injected into the ear vein. Mar. 2, 2.0 cc., in two doses. Hemolytic power: Mar. 10, 0.001 cc., complete hemolysis of sheep corpuscles; 0.0008 cc., incomplete hemolysis.

[3] Quantity of sediment.

Rabbit 6.—Weight 1,155 gm. Feb. 24, 2.5 cc. of hen red corpuscles injected intravenously. Mar. 2, 2.0 cc., in two doses. Hemolytic power: Mar. 10, 0.0025 cc., complete hemolysis of sheep corpuscles; 0.002 cc., almost complete hemolysis.

Rabbit 7.—Weight 1,510 gm. Feb. 24, 1.5 cc. of hen red corpuscles injected intravenously. Mar. 2, 2.5 cc. Hemolytic power: Mar. 10, 0.00125 cc., complete hemolysis of sheep corpuscles; 0.0011 cc., almost complete hemolysis.

TABLE I.

No. of rabbit.	1	2	3	4	5	6	7
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
Hemolytic power of serum.....	0.01	0.001	0.002	0.00125	0.001	0.0025	0.00125

In Table I are indicated the minimal doses of serum of each rabbit that gave complete hemolysis of sheep red corpuscles.

The protocols show that hen red corpuscles contain the same heterogeneous antigen-producing hemolysin against sheep red corpuscles as the organs of this animal. At any rate the quantity of heterogeneous antigen in the red corpuscles of the hen is not less than that in its organs, and the heterogeneous sera obtained possess a high hemolytic power. I believe that these experiments invalidate the law of Doerr and Pick.

The organs as well as the red corpuscles of the same animal may contain heterogeneous antigen; if it is not found, however, in the red corpuscles of mammals whose organs possess it, this may be attributed to the absence of a nucleus containing a large amount of nucleoprotein of cellular body. It is possible that the heterogeneous antigen may be found also in the red corpuscles of the pigeon, turtle, and toad. According to Amako (10), the organs of these animals also contain heterogeneous antigens.

The paradoxical nature of heterogeneous hemolytic sera is evident from these experiments. This refers to the fact that the heterogeneous sera possessing a high dissolving action on sheep red corpuscles contain a very small quantity of hemolysin against the red corpuscles of the species whose organs were used as antigen.

1 cc. of the hemolytic sera used contained about 1,000 hemolytic units against sheep red corpuscles (Table I), but they were almost

inactive against hen red corpuscles from which they were produced[4] (Table II).

TABLE II.

No of rabbit.	0.1 cc. of serum.	0.02 cc. of serum.	0.01 cc. of serum.
2	Tr.*	Tr.	Tr.
3	I.	"	"
4	Tr.	"	"
5	"	"	"
6	"	"	"
7	"	"	"

*In the tables Tr. indicates trace of hemolysis; I., incomplete hemolysis; Sl., slight hemolysis; N., no hemolysis; C., complete hemolysis.

The experiment was made as follows: 3 cc. of liquid in each tube, in the presence of 0.1 cc. of guinea pig complement (which alone is unable to hemolyze hen red corpuscles) and of 1 cc. of 5 per cent suspension of hen red corpuscles washed three times.

The almost complete absence of hemolysins in the red corpuscles of the hen which had furnished the antigen, and the formation of hemolysin against the red corpuscles of an animal of another species (sheep) disprove more forcibly the theory of the specificity of antibodies than did the phenomenon of the simultaneous presence of the specific antigen and an accidental one (common to different animals and bacteria) inside the cell.

[4] A number of sera from normal rabbits were treated. Their power of dissolving hen red corpuscles was as follows:

Normal rabbits.	0.1 cc.	0.3 cc.	0.5 cc.	0.7 cc.	1.0 cc.
1	I.	I.	I.	Almost C.	Almost C.
2	"	"	"	I.	I.
3	Tr.	Tr.	Tr.	Tr.	"
4	I.	I.	I.	I.	Almost C.
5	"	"	"	"	I.

Therefore, 0.1 cc. given to four normal rabbits produced even a stronger hemolysis than when given to immunized animals. Yet 0.02 and 0.01 cc. never showed any trace of hemolysis. This fact did not allow me to disprove the presence of immune hemolysins against hen red corpuscles.

These facts were discovered by Forssman, but neither he nor the other workers who studied the problem of heterogeneous hemolysins insisted upon it sufficiently. Forssman noticed that his sera produced by rabbits which had been immunized with guinea pig organs contained in 1 cc. 200 and 1,000 hemolytic units against sheep red corpuscles, and only 25 to 30 units against guinea pig red corpuscles; but he stated that the hemolysin against sheep red corpuscles was the principal one, and that the other, against guinea pig red corpuscles, was accessory. In my experiments the accessory hemolysin has not been definitely discovered, because even 0.1 cc. was unable to hemolyze completely one unit of hen red corpuscles (Table II).

Thus the fact established by Forssman becomes particularly salient in connection with the heterogeneous sera that I obtained. Moreover, the use of animal organs for immunization may suggest the objection that the absence or slight formation of homologous hemolysins was due to the organ specificity of the antigen. With red corpuscles the question of organ specificity is excluded.

The question therefore arises: What if the antigenic action of hen red corpuscles were only an exception? Could we not explain the absence of specific hemolytic antigen in hen red corpuscles by their having lost all receptors of their species, such as, for instance, the crystalline of adult animals? The following experiments contradict this hypothesis. Hen red corpuscles derived from specific hemolytic antigen possess specific agglutinogens. After immunization of rabbits a great quantity of agglutinin to hen red corpuscles was formed in the serum (Table III).

TABLE III.

No. of rabbit.	0.01 cc. of serum.	0.002 cc. of serum.	0.001 cc. of serum.	0.0002 cc. of serum.	0.0001 cc. of serum.	0.00005 cc. of serum.	0.00002 cc. of serum.	0.0000125 cc. of serum.	0.00001 cc. of serum.	0.000005 cc. of serum.
2	+	+	+	+	+	+	+	+	+	−
3	+	+	+	+	+	Sl. +	−			
4	+	+	+	+	+	Sl. +	−			
5	+	+	+	+	+	+	+	+	+	−
6	+	+	+	+	+	+	+	+	+	−
7	+	+	+	+	+	+	+	+	+	−

0.2 cc. of a 5 per cent suspension of hen red corpuscles was added to 1 cc. of serum dilution; the tubes were kept at 37° C. for 2 hours and then on ice until morning.

The sera in question contain not only heterogeneous hemolysins but also heterogeneous agglutinins (Table IV).

TABLE IV.

No. of rabbit.	0.1 cc. of serum.	0.02 cc. of serum.	0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.	0.00125 cc. of serum.	0.001 cc. of serum.
2	+	+	+	+	+	Sl. +	−
3	+	+	+	+	−		
4	+	+	+	+	+	Sl. +	−
5	+	+	+	+	+	−	
6	+	+	+	+	Sl. +	−	
7	+	+	+	+	−		

The experiment with sheep red corpuscles was carried out like that of Table III.

Forssman and Hintze (11) several times found small quantities[5] of agglutinin to sheep red corpuscles in sera of rabbits immunized with guinea pig organs. Doerr and Pick (4) state that most of the heterogeneous sera that they obtained from rabbits immunized with horse or guinea pig organs were devoid of hemagglutinins; in another place (5) they state that all the sera produced by rabbits immunized with horse organs were devoid of agglutinins to sheep red corpuscles. Considering, however, that heterogeneous sera of rabbits immunized with hen red corpuscles always possess heterogeneous hemagglutinins, one must reject the statement of Doerr and Pick (4) who thought that the absence of agglutinins in heterogeneous sera was one of the characteristics distinguishing them from homologous sera. The difference in the results of Forssman's experiments and mine on the one hand, and Doerr and Pick's on the other, may be explained by the fact that some heterogeneous sera (immunized with horse organs) are totally devoid of hemagglutinins, some possess them accidentally (immunized with guinea pig organs), and some, such as the sera that are the special objects of this investigation, always contain agglutinins to sheep red corpuscles. The latter sera, produced by rabbits immunized with hen red corpuscles, possess less heterogeneous agglutinins, however, than heterogeneous hemolysins (Tables I and IV).

[5] The quantity is not exact.

Heterogeneous sera of rabbits, immunized with hen red corpuscles, do not contain a hemolysin against the red corpuscles of the ox, like the heterogeneous sera studied by earlier authors (Table V).

TABLE V.

No. of rabbit.	0.1 cc. of serum.	0.02 cc. of serum.	0.01 cc. of serum
2 3 4 5 6 7	No hemolysis.	No hemolysis.	No hemolysis.

The experiment with red corpuscles was carried out like that of Table I.

Like the heterogeneous sera studied earlier, the hemolytic sera of rabbits immunized with hen red corpuscles are toxic to guinea pigs and kill them, with the clinical picture of anaphylactic shock.

Serum 2.—A guinea pig weighing 265 gm. was injected with 1 cc. of serum, into the jugular vein. Death followed in 5 minutes. 30 seconds after the beginning of the experiment the guinea pig appeared to have a chill; convulsions followed and the animal fell on its side; in this position were observed tonic and clonic convulsions of the extremities, shortness of breath, and loss of reflexes. A foaming serous liquid, not tinged with blood, came from the mouth and nostrils. Autopsy: thrombus in heart cavity and vessels, and in the vena cava inferior; the lungs were inflated, covering a large part of the heart; no lung hemorrhage; the lungs on section showed a foaming serous liquid.

A guinea pig weighing 170 gm. received 0.5 cc. of serum into the jugular vein. Death followed in 6.5 minutes. There was the same clinical and anatomical picture as in the preceding case.

A guinea pig weighing 170 gm. received 0.3 cc. of serum into the jugular vein. Death followed after 5.5 minutes. The same clinical and anatomical picture.

A guinea pig weighing 170 gm. received 0.1 cc. of serum into the jugular vein. The only effect observed was a brief chill.

Serum 3.—A guinea pig weighing 185 gm. received 1 cc. of serum into the jugular vein. Death followed after 7 minutes. Clinical picture as in the preceding cases. Autopsy: thrombus in the right heart and vessels; lungs inflated, edematous, slight hemorrhage.

A guinea pig weighing 150 gm. received 0.3 cc. of serum into the jugular vein. The only effect was a brief chill.

Serum 4.—A guinea pig weighing 260 gm. received 1 cc. of serum into the jugular vein. Death after 3.5 minutes. Clinical picture as in the preceding cases but without foaming liquid. The same anatomical changes as in the animals killed by Serum 2, but the lung inflation was insignificant and there was no edema.

A guinea pig weighing 175 gm. received 0.5 cc. of serum into the jugular vein. Death followed after 5 minutes. The same clinical picture as in the animals killed by Serum 2. Autopsy: thrombus only in the right heart and vessels; lungs slightly inflated, edematous.

A guinea pig weighing 170 gm. received 0.3 cc. of serum into the jugular vein, with no effect.

Heterogeneous hemolysins of rabbits immunized with hen red corpuscles can be bound with their antigen (Table VI).

TABLE VI.

Date.	No. of serum.	0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.	Experiment of binding hen red corpuscles with homologous serum No 8.		
					0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.
Mar. 5	2*	N.	N.	N.	C.	I.	Tr.
	4*	"	"	"			
	5†	I.	Tr.	Tr.			
" 10	6†	Tr.	"	"	"	Almost C.	I.
	7†	"	"	"			

* A suspension was used of 3 cc. of hen red corpuscle sediment in 10 cc. of physiological salt solution.

† A suspension was used of 3 cc. of hen red corpuscle sediment in 20 cc. of physiological salt solution.

The experiment was carried out as follows: A suspension of hen red corpuscles was freed of serum by washing and was mixed with equal parts of heterogeneous hemolysin prepared in the proportion of 1:50; the mixture was kept at 37°C. for 1 hour, then centrifuged. The hemolytic power of the liquid against hen red corpuscles was tested at 37° C. during 2 hours.

The kidneys of the hen, which according to Doerr and Pick contain heterogeneous antigens, also bind the hemolysins in question, but less completely (Table VII).

TABLE VII.

Date.	No. of rabbit.	0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.	Experiment of binding suspension of hen kidney with homologous serum No. 8.		
					0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.
Mar. 12	5	C.	Tr.	Tr.	C.	Almost C.	I.
	6	I.	"	"			
	7	"	"	"			

The experiments were carried out in the same way as those of Table VI. The kidney suspension was prepared in the proportion of 3 gm. to 20 cc. of physiological salt solution.

The antigen found in guinea pig organs and producing heterogeneous hemolysin against sheep red corpuscles reveals inconstant relations in binding hemolysins produced by rabbits immunized with hen red corpuscles (Table VIII).

TABLE VIII.

Date.	No. of rabbit.	0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.	Experiment of binding suspension of guinea pig kidney with homologous serum No. 8.		
					0.01 cc. of serum.	0.003 cc. of serum.	0.002 cc. of serum.
Mar. 5	2	C.	C.	I.	C.	I.	Tr.
	4	"	I.	Tr.			
" 10	5	I.	Tr.	"	"	Almost C.	I.
	6	Tr.	"	"			
	7	"	"	"			

The experiments were carried out in the same way as those of Table VII.

The experiments given in Table VIII show that the heterogeneous antigen of guinea pig kidneys sometimes can be bound with the hemolysin in question in the same degree as the antigen of hen red corpuscles (Nos. 5, 6, and 7); in other cases the binding is very weak (No. 4) or is entirely absent (No. 2).

The reason for this inconstant binding of the heterogeneous hemolysin in question with a suspension of guinea pig kidneys is not clear.

The circumstance that the antigen used was not the same one which had generated the hemolysin may be of some importance. Further, it is possible that we have to deal with a phenomenon noticed by Bail and Margulies (12), who state that in 40 per cent of the experiments guinea pig organs do not bind the hemolysin at all or do so weakly. My experiments in binding the homologous hemolysin No. 8 (the right hand columns of Tables VI, VII, and VIII) with different heterogeneous antigens agreed with the observations of previous authors because the binding between that homologous hemolysin and the antigens of hen red corpuscles, and hen and guinea pig kidneys was not significant.

The experiments in Table IX were undertaken to ascertain the respective part of absorption and specific binding of the antibodies contained in the sera used.

TABLE IX.

No. of serum.	Sera after incubation with hen red corpuscles.			Sera after incubation with suspension of guinea pig kidney.					
	0.01 cc. of serum.	0.002 cc. of serum.	0.001 cc. of serum.	0.01 cc. of serum.	0.002 cc. of serum.	0.001 cc. of serum.	0.0002 cc. of serum.	0.0001 cc. of serum.	0.00005 cc. of serum.
2	+	Sl. +	−	+	+	+	+	+	Sl. +
4	+	Sl. +	−	+	+	+	Sl. +	−	−
5	+	−	−	+	+	+	+	+	Sl. +
6	Sl. +	−	−	+	+	+	+	+	−
7	+	−	−	+	+	+	+	+	−

The sera used were prepared in the same way as in the experiments of Tables VI and VII. 1 cc. of the serum dilution was mixed with 0.2 cc. of a 5 per cent suspension of hen red corpuscles; the tubes were shaken and kept at 37° C. for 2 hours, then on ice until morning.

In this way was determined the quantity of hemagglutinins remaining after treating the serum (1) with hen red corpuscles possessing both heterogeneous antigen (hemolysin against sheep red cells) and homologous specific antigen (hemagglutinogen), and (2) with a suspension of guinea pig kidneys containing only heterogeneous antigen, producing a hemolysin against sheep red corpuscles.

From the quantity of hen hemagglutinins left, we are able to determine the relative importance of absorption (treating with guinea pig

kidneys deprived of hen agglutinogen) and of specific elective binding between antigen and antibody (treating with suspension of hen red corpuscles).

From Table III and the right hand columns of Table IX the intensity of the phenomenon of absorption is seen, as it has the power to deprive the serum of nine-tenths of its agglutinins. From Table III and the left hand columns of Table IX one can infer that the elective affinity between antigen and antibody is very great; it leaves in the serum only 0.001 (Nos. 2, 5, and 7), 0.002 (No. 6), and at the most 0.005 (No. 4) of its antibodies. Therefore, comparing the right and left hand columns of Table IX, we must assign the most important part to the specific elective affinity between antigen and antibodies; however, we should not lose sight of the part that non-specific absorption plays in the presence of a suspension of animal organs.

It is evident that heterogeneous hemolysins have been obtained by means of non-toxic antigen, such as hen red corpuscles injected into the veins of rabbits. This refutes the theory of Friedberger and Schiff who attributed the antigenic value of a suspension of organs to its toxic character.

The following experiments prove that the connective tissue of animals (guinea pigs) contains heterogeneous sheep antigen. The fatty tissue enveloping the intestines and kidneys of an exsanguinated guinea pig was rubbed and washed three times with physiological salt solution to remove every trace of serum. In this condition the adipose tissue becomes a strictly connective tissue deprived of any other textile element of the organism, except the nerve ramifications.

Rabbit A.—Weight 1,590 gm. Oct. 26, 0.3 gm. of fat injected into the vein and 1 gm. into the abdomen. Nov. 5, 1 gm. of fat injected into the abdomen. Hemolytic power of the serum against sheep red corpuscles: Nov. 5, 0.01 cc., complete hemolysis; 0.003 cc., incomplete hemolysis; Dec. 13, 0.01 cc., complete hemolysis; 0.0025 cc., incomplete hemolysis.

Rabbit B.—Weight 1,260 gm. Jan. 8, 0.2 gm. of fat injected into the vein and 0.8 gm. into the abdomen. Jan. 17, 1.5 gm. of fat injected into the abdomen. Hemolytic power: 0.01, 0.003, and 0.0025 cc., complete hemolysis of sheep corpuscles; 0.002 cc., almost complete hemolysis.

Bail and Margulies supposed that the connective tissue possessed heterogeneous hemolytic antigen but quote no experiments to prove

it. The experiments described above fully demonstrate that the connective tissue possesses heterogeneous hemolytic antigen.

Bail and Margulies not only thought that the connective tissue possessed heterogeneous antigen; they supposed that the faculty of producing heterogeneous hemolysins, proper to organs of animals, was entirely due to the contents of heterogeneous antigen in the stroma of connective tissue. I undertook some experiments hoping to solve the problem by producing degeneration[6] of the parenchymatous cells of the organ (kidney). Rabbits were immunized with degenerated guinea pig organs, as it was thought that a strong diminution in the production of heterogeneous hemolysin would disprove the hypothesis of Bail and Margulies, because it would prove the antigenic value of the epithelial cells of the organ, which were excluded by degeneration. Unfortunately, the method failed. First, the animals withstood the immunization badly, and most of them died. The decrease, therefore, in the production of antibodies may have been due to cachexia of the rabbits rather than to any loss of antigen in the parenchymal cells injected. Besides, the histological picture of the kidneys after such injections was a very inconstant one; degeneration or necrosis was present in the convoluted tubules only, not in the straight ones; all the epithelial elements of the kidney became degenerated, and only the stroma and the glomeruli were morphologically unaltered. It is therefore impossible to draw any conclusion.

As they believe that only the connective tissue possessed heterogeneous antigen, Bail and Margulies undertook to prove that this antigen should be found in the organs of even those animals which were supposed not to have it, because the connective tissue, being a slightly differentiated formation, possesses identical characteristics in every animal. If, nevertheless, many animals never reveal heterogeneous antigen, that may be explained by the serum coming into contact with the heterogeneous antigen and binding it. Bail and Margulies believe that they succeeded in proving this hypothesis by their experiments. They bound heterogeneous antigens in organs of animals which possess them, by sera of animals which do not. But the results of the method they adopted are inconstant, as is evident from

[6] 1 cc. of saturated sublimate solution was injected subcutaneously.

the tables and protocols of their article. It therefore seemed necessary to choose a more certain way, such as the immunization of an animal with a mixture of an organ containing heterogeneous antigen with the serum of an animal whose organs do not possess heterogeneous antigen.

The procedure was as follows: Guinea pig kidneys were ground fine in physiological salt solution, 10 gm. of organ to 14 cc. of solution. The suspension was filtered through muslin and was mixed with rabbit serum, 2 cc. of suspension to 4 cc. of serum. The mixture was kept at 37°C. for 2 hours and was then injected into rabbits.

Rabbit A.—Weight 1,350 gm. Oct. 13, 3 cc. injected intravenously. Oct. 20, 5 cc. into the abdomen. Hemolytic power: Oct. 20, 0.006 cc., complete hemolysis of sheep corpuscles; 0.005 cc., incomplete hemolysis; Oct. 29, 0.002 cc., complete hemolysis; 0.0016 cc., incomplete hemolysis.

Rabbit B.—Weight 1,860 gm. Oct. 26, 10 cc. injected into the abdomen. Hemolytic power: Nov. 5, 0.003 cc., complete hemolysis of sheep corpuscles; 0.0025 cc., traces of hemolysis.

These experiments prove that a suspension of guinea pig organs conserves the faculty of producing hemolysins after a contact of 2 hours with the serum of a rabbit whose organs are deprived of heterogeneous antigen. In consequence, the serum is unable to deprive the organs of animals of their hemolytic antigen by its contact, and the absence of heterogeneous antigen in some species of animals cannot be explained by the neutralizing power of its serum.

SUMMARY.

Red corpuscles with nuclei contain heterogeneous sheep antigen when the organs of the same animal possess it.

The sera in question contain not only heterogeneous hemolysins but also heterogeneous agglutinins.

The hemolytic sera of rabbits immunized with hen red corpuscles are toxic to guinea pigs and kill them, the clinical picture being that of anaphylactic shock.

Heterogeneous hemolysins are obtained by means of non-toxic antigen (hen red corpuscles injected into veins of rabbits). This fact refutes the theory of Friedberger, who attributed the antigenic power of a suspension of organs to its toxic character.

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THE EFFECTS OF SERUM TREATED WITH AGAR.

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INTRODUCTION.

Bordet,[1] in 1913, showed that by adding to normal guinea pig serum a small quantity of a semifluid suspension of agar in physiological solution of sodium chloride a liquid was obtained, which, on intravenous injection into guinea pigs, caused typical anaphylactic intoxication. The mixture of serum and agar was kept at 37°C. for 2 hours, and then subjected to rapid centrifugalization to separate out the grayish sediment of the agar. The symptoms produced in the animals were tremors, dyspnea, micturition, and death after a few minutes. The autopsy which was performed immediately showed dilatation of the lungs with hemorrhagic areas varying in size and number, persistence of the heart beat, and a marked retardation of the coagulation of the blood. This picture is identical with that of anaphylaxis caused by the injection of antigen into a sensitized animal. Serum treated with agar acts in the same manner as anaphylatoxin (Friedberger). Of course, the intravenous injection of physiological salt solution kept for 2 hours at 37°C. in the presence of the suspension of agar, and freed from the sediment by centrifugalization, is innocuous to the guinea pig.

If agar is added to serum previously heated to 56°C., and then treated in the manner described above, the serum will be as harmless as the normal control serum not kept for 2 hours at a temperature of 37°C. in contact with the diluted agar.

Nathan[2] has shown that it is sufficient to add to 5 cc. of normal guinea pig serum 0.1 cc. of a suspension of 0.5 per cent agar, that is, 0.5 mg. of agar, to make this serum toxic after a minimum period of 1 to 1½ hours at 37°C. These

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[1] Bordet, J., Le mécanisme de l'anaphylaxie, *Compt. rend. Soc. biol.*, 1913, lxxiv, 225; Gélose et anaphylatoxine, *ibid.*, 1913, lxxiv, 877; Qu'est-ce que l'anaphylaxie? *Ann. et Bull. Soc. roy. sc. méd. et nat. Bruxelles*, 1913, lxxi, 25.

[2] Nathan, E., Ueber Anaphylatoxinbildung durch Agar, *Z. Immunitätsforsch., Orig.*, 1913, xvii, 478.

facts have been confirmed by Friedberger,[3] Haren,[4] Kraus and Kirschbaum,[5] Loewit and Bayer,[6] Tchernoroutzky,[7] and others.

For agar, a product which always contains relatively large quantities of nitrogen, pararabin may be substituted; that is, a part of the carbohydrates of agar. This pararabin of agar is practically free from nitrogen, containing only 0.23 per cent at the most. Guinea pig serum kept 2 or 3 hours at 37°C. in contact with this pararabin, on intravenous injection into the guinea pig, causes the same syndrome as serum treated with agar. Previous heating to 56°C. for 20 to 30 minutes prevents the serum from becoming toxic in the presence of the pararabin.[8]

Similar results are obtained by treating the serum with starch,[9] a suspension of inulin,[10] or a sodium compound of pectin free from nitrogen.[11] On the other hand, a solution of inulin does not give to the serum the properties of anaphylatoxin. The physical constitution of the product with which the serum is kept in contact at 37°C. for an adequate period seems to be of importance. For the moment, however, we shall leave this point.

Notwithstanding the marked similarity of symptoms observed on the one hand by reinjecting heterologous serum into a guinea pig sensitized 2 to 3 weeks before by this serum, and on the other hand by introducing guinea pig serum treated with agar or pararabin into the jugular vein of a normal guinea pig, the identity of the two phenomena is not fully established.

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[3] Friedberger, E., Handelt es sich bei der Anaphylatoxinbildung aus Agar-Agar nach Bordet um eine physikalische Absorptionswirkung, *Z. Immunitätsforsch., Orig.*, 1913, xviii, 323.

[4] Haren, P., Ueber die Giftigkeit arteigenen Serums und die Anaphylatoxinbildung aus Agar und Gelatine, *Z. Immunitätsforsch., Orig.*, 1913–14, xx, 673.

[5] Kraus, R., and Kirschbaum, P., Zur Frage der anaphylaktischen Vergiftung, *Wein. klin. Woch.*, 1913, xxvi, 783.

[6] Loewit, M., and Bayer, G., Die Abspaltung von Anaphylatoxin aus Agar nach Bordet, *Arch. exp. Path. u. Pharm.*, 1913, lxxiv, 478.

[7] Tchernoroutzky, Sur l'anaphylatoxine de Bordet, *Compt. rend. Soc. biol.*, 1913, lxxiv, 1213.

[8] Bordet, J., and Zunz, E., Production d'anaphylatoxine dans le sérum traité par de l'agar épuré de son azote (pararabine), *Z. Immunitätsforsch., Orig.*, 1914–15, xxiii, 42.

[9] Nathan, Ueber Anaphylatoxinbildung durch Stärke, *Z. Immunitätsforsch., Orig.*, 1913, xviii, 636.

[10] Nathan, Ueber Anaphylatoxinbildung durch Inulin, *Z. Immunitätsforsch., Orig.*, 1914–15, xxiii, 204.

[11] Kopaczewski, W., and Mutermilch, S., Sur l'origine des anaphylatoxines, *Compt. rend. Soc. biol.*, 1914, lxxvi, 782; *Z. Immunitätsforsch., Orig.*, 1914, xxii, 539.

Other analogies between these two groups of phenomena have been noted. A few days after the first parenteral injection of heterologous serum there appears, in the plasma of the animals submitted to the treatment, a proteolytic power for the sensitizing proteins.[12] More exactly, amino nitrogen is set free if the plasma of the sensitized animal is kept for 2 hours at 38°C. in the presence of the sensitizing serum. This amino nitrogen arises from the division of the peptic radicals, CONH, which constitute the essential part of the protein molecules and their immediate derivatives, proteoses, peptones, and peptides. This special proteolytic power acquires its maximum of intensity at the time when the sensitized animal reaches the anaphylactic state; that is, at the period during which the reinjection of the sensitizing serum produces symptoms of anaphylactic shock. This period, for example, in dogs after a sensitizing intravenous injection of ox serum is 15 days.[13]

If guinea pig serum is kept for 2 or 3 hours at 38°C. in the presence of agar or pararabin, disintegration of the proteins takes place as in the preceding case. This phenomenon does not occur[14] when the serum has been kept 20 or 30 minutes at 56°C. In this case also, a remarkable agreement is observed between the results obtained with animals sensitized by serum in a state of anaphylaxis, and the results obtained after treatment of normal guinea pig serum with agar or pararabin.

The importance of these experiments for the comprehension of anaphylaxis and its bearing on immunity will be readily conceded, although it would be premature to make any assertions on this subject. The study of the effects of serum treated with agar must first be extended to other species of animals. In collaboration with Bordet, one of us undertook these tests with rabbits and dogs, but the investigations were interrupted before their completion. Nevertheless, the results of the first experiments with rabbits tend to confirm the observations made with guinea pigs, considering the differ-

[12] Abderhalden, E., Abwehrfermente, Berlin, 4th edition, 1914, 62 and following. Pfeiffer, H., Das Problem der Eiweissanaphylaxie, Jena, 1910; Die Arbeitsmethoden bei Versuchen über Anaphylaxie, *Abderhalden's Handb. biochem. Arbeitsmethoden*, 1911, v, 525. Zunz, E., Recherches sur le pouvoir protéoclastique du sang au cours de l'anaphylaxie (1re à 3e communications), *Z. Immunitätsforsch., Orig.*, 1913, xvii, 279.

[13] Zunz, E., and György, P., Recherches sur le pouvoir protéoclastique du sang au cours de l'anaphylaxie, (4e communication), *Z. Immunitätsforsch., Orig.*, 1914–1915, xxiii, 402.

[14] Bordet and Zunz, Le sérum traité par l'agar épuré de son azote (pararabine) se protéolyse-t'-il, *Z. Immunitätsforsch., Orig.*, 1914–15, xxiii, 49.

ent symptoms of anaphylactic shock produced in the two species of animals.

Arthus[15] was able, in the course of numerous experiments, to assure himself of the innocuousness of intravenous injections of considerable quantities of horse serum in normal rabbits. However, the introduction of horse serum into the circulatory system of rabbits, which had first received subcutaneous serum injections, led to a series of general symptoms; fall of arterial pressure, anuria, accelerated respiration, and expulsion of a considerable number of scybala. It is easy to determine from the technique used by Arthus whether or not one is dealing with symptoms of anaphylactic shock.

We also employed this method to determine whether or not horse serum kept 2 hours at 38°C. on agar and treated in the manner indicated by Bordet in his experiments with guinea pigs, produces anaphylactic shock in normal rabbits.

Technique.

The suspension of agar was prepared as follows: 1 gm. of agar was dissolved by heating in 200 cc. of physiological salt solution. This solution was distributed in test-tubes in portions of 10 cc., sterilized, and sealed. Upon cooling, a soft jelly is formed. Vigorous shaking will transform this jelly into a homogeneous suspension of agar which keeps well.

To 50 cc. of horse serum were added 10 cc. of a suspension of agar; *i.e.*, five parts of serum to one part of agar. In addition we prepared the four following control mixtures:

1. 50 cc. of fresh horse serum plus 10 cc. of physiological salt solution.
2. 50 cc. of physiological salt solution plus 10 cc. of a suspension of 0.5 per cent agar.
3. 50 cc. of horse serum kept for 30 minutes at 56°C. plus 10 cc. of physiological salt solution.
4. 50 cc. of horse serum kept for 30 minutes at 56°C. plus 10 cc. of a suspension of 0.5 per cent agar.

The five mixtures were kept in well corked Erlenmeyer flasks for 2 hours at 38°C. At the end of this period, each solution was submitted first to rapid centrifugalization and then to filtration. Thus the agar contained in the test mixture and in the second and fourth of the control mixtures was removed.

The liquids were injected into rabbits in the marginal vein of the ear. The

[15] Arthus, M., La séro-anaphylaxie du lapin, *Arch. de physiol.*, 1908–09, vii, 471; 1910, ix, 156.

respiration was registered by Verdin's recording tambours, connecting with a Marey tambour; and the carotid pressure by Verdin's registering hemadynamometer. Franck's cannula was employed. As an anticoagulant a solution of 65 gm. of sodium bicarbonate and 15 gm. of magnesium sulphate in 1,000 cc. of water was employed.

In order to estimate the coagulability we discarded the first few cubic centimeters of carotid blood, contaminated by mixture with the anticoagulant solution. The blood is collected from the carotid directly into a porcelain vessel, without touching the wound. The time necessary for the formation of the first fibrin floccules and for complete coagulation was recorded.

EXPERIMENTAL.

We performed four series of tests, each with a fresh sample of horse serum. In the first series we prepared but one control mixture, of normal serum and physiological salt solution.

First Series of Experiments.

Experiment 1.—A rabbit weighing 1,800 gm. received an intravenous injection of 10 cc. of control serum (normal horse serum plus physiological salt solution, kept 2 hours at 38°C.); 8 minutes later 10 cc. of the filtered test mixture (serum plus suspension of agar) were injected. This dose corresponds to 5.56 cc. per kilo of control serum and of serum treated with agar. Table I gives the results of arterial pressure and respiratory rate in the experiments.

TABLE I.

Injection of Normal Horse Serum and Physiological Salt Solution (Control).

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	9.0	60	4	8.7	67
½	9.0	68	5	8.7	57
1	8.9	65	6	8.6	55
2	8.9	63	7	8.8	62
3	8.8	69			
Injection of Filtrate of Serum Treated with Agar.					
½	9.0	63	13	7.6	61
1	9.0	64	14	7.7	60
2	8.6	61	15	7.7	63
3	8.2	58	16	7.4	58
4	8.3	62	17	7.3	61
5	8.2	61	18	7.2	63
6	8.4	62	19	7.3	60
7	8.2	67	20	7.2	58
8	8.2	64	21	7.3	60
9	8.1	54	22	7.3	58
10	8.0	55	23	7.4	53
11	8.0	61	24	7.5	61
12	7.6	62	25	7.5	58

The intravenous injection of control serum caused a gradual slow fall in the arterial blood pressure. In 6 minutes it fell 0.4 cm. of mercury, and then tended to rise again. After injecting serum treated with agar the fall in pressure was also gradual, but much greater. It amounted to 1.8 cm. of mercury in 18 minutes. The arterial pressure remained stationary for a time, and rose again slowly. The respiration showed no distinct changes after either the first or second injection. There was no defecation.

Blood taken from the carotid 26 minutes after the second injection showed the first fibrin formation at the end of 18 minutes. Coagulation was complete 4 minutes later; that is, 22 minutes after drawing the blood.

Experiment 2.—A rabbit weighing 1,750 gm. received intravenously an injection of 5 cc. of control serum (normal horse serum plus physiological salt solu-

tion kept 2 hours at 38°C.). 8 minutes later 5 cc. of the filtered test mixture (serum plus agar suspension) were given. This dose corresponds to 2.86 cc. per kilo of control serum and of serum treated with agar. (Table II.)

TABLE II.

Injection of Normal Horse Serum and Physiological Salt Solution (Control).

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	9.6	62	4	9.6	68
½	9.9	68	5	9.7	65
1	9.9	60	6	9.6	69
2	9.8	67	7	9.6	65
3	9.6	67			
Injection of Filtrate of Serum Treated with Agar.					
½	9.9	68	11	9.0	56
1	9.6	65	12	9.0	58
2	9.3	68	13	9.2	62
3	9.1	62	14	9.3	65
4	9.0	63	15	9.3	65
5	9.0	57	16	9.1	67
6	8.9	67	17	9.1	63
7	8.8	62	18	9.2	63
8	8.8	63	19	9.3	66
9	9.0	57	20	9.4	60
10	9.0	62	21	9.4	65

The injection of control serum did not lead to a noticeable modification of the respiratory rate or of the arterial pressure, except for a slight temporary increase of the pressure, 0.3 cm. of mercury. The injection of serum treated with agar had also no effect on the respiration. The arterial pressure rose temporarily 0.3 cm. of mercury, then it fell in 7 minutes 0.8 cm. of mercury, as compared with the initial level. No defecation took place after either injection.

Blood taken from the carotid 22 minutes after the injection of serum treated with agar showed the first fibrin formation at the end of 8 minutes; coagulation was complete in 7 minutes more; that is, 15 minutes after the withdrawal of the blood.

In this first series of experiments we noted an effect of the serum treated with agar on the arterial pressure. This effect was very

slight with a dose of 2.86 cc. of serum per kilo, and more pronounced with 5.56 cc. per kilo.

Second Series of Experiments.

Experiment 3.—A rabbit weighing 1,620 gm. received an intravenous injection of 5 cc. of serum treated with agar; that is, 3.09 cc. per kilo. (Table III.)

TABLE III.

Injection of Filtrate of Serum Treated with Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	9.9	51	17	6.4	53
½	9.9	50	18	6.4	53
1	9.9	45	19	6.4	52
2	8.1	45	20	6.3	48
3	7.9	50	21	6.4	50
4	7.5	55	22	6.5	50
5	6.4	49	23	6.5	50
6	5.8	49	24	6.4	50
7	5.8	48	25	6.5	61
8	6.5	59	26	6.6	55
9	6.3	47	27	6.7	52
10	6.4	50	28	6.6	55
11	6.6	47	29	6.7	51
12	6.6	48	30	6.7	48
13	6.3	56	31	6.8	48
14	6.2	60	32	6.4	48
15	6.2	53	33	6.6	53
16	6.3	58			

After the injection of serum which had been kept for 2 hours at 38°C. in contact with agar, the arterial pressure fell 4.1 cm. of mercury in 6 minutes. The pressure rose again 0.7 cm. 8 minutes after the injection and remained, with some variation, near this level until the end of the experiment. The respiration did not undergo any actual modifications. There was an expulsion of thirty scybala shortly after the injection.

The carotid blood, drawn 34 minutes after the injection, showed the first fibrin formation at the end of 13 minutes; coagulation was complete 4 minutes later, or 17 minutes after the drawing of blood.

Experiment 4.—A rabbit weighing 1,810 gm. was injected intravenously successively with 5 cc. of the following liquids, 2.76 cc. of each per kilo. The order of injections was as follows:

1. Serum and physiological salt solution.
2. Filtrate of agar and physiological salt solution.
3. Filtrate of serum, which had been heated to 56°C., and of agar.
4. Serum previously heated to 56°C. and physiological salt solution.
5. Filtrate of serum and agar.

About half a minute was required for each injection. (Table IV.)

TABLE IV.

Injection of Serum and Physiological Salt Solution (Control).

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	10.4	83	4	10.6	89
½	12.2	98	5	10.3	100
1	11.6	89	6	10.2	112
2	11.4	92	7	10.3	99
3	11.1	90	8	10.6	104
Injection of Filtrate from Control Mixture of Agar and Physiological Salt Solution.					
½	10.0	102	5	9.6	92
1	10.0	98	6	9.4	94
2	10.1	95	7	9.3	83
3	9.8	98	8	9.4	84
4	9.7	95	9	9.6	78
Injection of Filtrate of Serum Heated to 56° C. and Agar (Control).					
½	9.7	77	5	9.0	56
1	9.6	76	6	9.0	67
2	9.0	62	7	9.0	67
3	9.0	52	8	9.3	68
4	8.9	54	9	9.5	70
Injection of Serum Heated to 56°C. and Physiological Salt Solution (Control).					
½	9.6	72	5	9.7	73
1	9.7	70	6	9.7	75
2	9.8	69	7	9.6	74
3	9.8	75	8	9.6	72
4	9.7	76			

TABLE IV—*Concluded.*

Injection of Filtrate of Serum Treated with Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
½	9.8	76	14	7.0	95
1	9.6	83	15	7.0	97
2	8.8	81	16	6.7	109
3	7.1	68	17	6.4	107
4	7.6	67	18	5.0	102
5	7.6	63	19	5.6	111
6	7.4	67	20	5.4	105
7	7.2	72	21	5.9	105
8	7.4	73	22	5.9	97
9	7.4	82	23	6.2	100
10	7.3	72	24	5.4	102
11	7.1	74	25	5.4	93
12	7.2	87	26	6.0	90
13	7.0	97	27	6.0	82

The arterial pressure rose considerably (1.8 cm. of mercury) immediately after the intravenous injection of the control serum kept 2 hours at 38°C. Subsequently it returned to its normal level and even fell somewhat (0.2 cm. of mercury) below normal toward the 6th minute, again rising slightly (0.2 cm. of mercury) above its original level 8 minutes after the injection.

The introduction into the circulation of physiological salt solution treated with agar caused an immediate fall of the arterial pressure, amounting to 1.3 cm. of mercury in 7 minutes. Then the pressure commenced to rise again.

The serum previously heated to 56°C., then treated with agar, caused a fall in the arterial pressure of 0.7 cm. of mercury in 4 minutes. The pressure rose slightly after the 7th minute following the injection.

This serum heated to 56°C., and kept for 2 hours at 38°C., had no actual effect on the arterial pressure, for we cannot ascribe to it the slight increase of 0.2 to 0.3 cm. of mercury, which would possibly also have taken place without the last injection, and which stopped before the arterial pressure had regained the level at which it was before the third injection.

The action on the arterial pressure of serum treated with agar was much more marked in the rabbit of Experiment 4 than that of the different control liquids. It consisted of a gradual fall. The pressure attained its minimum 18 minutes after the injection. At this moment it had fallen 4.8 cm. of mercury. Then it rose again about 1 cm. and remained at this level, with some fluctuations, until the end of the experiment.

If we compare the effects of the various intravenous injections in the rabbit of Experiment 4, and the results obtained in the rabbits of Experiments 3 and 4 after the intravenous injection of serum treated with agar, it is evident that this serum has acquired, after the treatment, the power of producing in a normal animal a fall of arterial pressure as marked as the one induced in a sensitized animal by intravenous injection of sensitizing serum. Prior to this treatment horse serum had different properties, since it caused a rise in the arterial pressure. After being exposed 30 minutes to 56°C. and treated with agar, the serum loses its power of causing any considerable fall in the arterial pressure. The inactivated serum has no effect on the pressure. It is of interest to note the relatively slight fall in pressure, caused by the physiological salt solution kept 2 hours at 38°C. on agar. This phenomenon is sometimes more pronounced, as seen in the third and fourth series of experiments.

We shall now consider the respiratory movements. The results obtained in the course of Experiment 4 may be summed up as follows: acceleration of respiratory rate was as great after the injection of serum treated with agar (maximum at the end of 19 minutes) as after the injection of control serum kept 2 hours at 38°C. (maximum at the end of 6 minutes); gradual slowing after injection with physiological salt solution previously treated with agar; slowing during 3 minutes following the injection of serum previously heated to 56°C., then treated with agar, and finally a return to the initial rate; no actual modifications after the injection of serum heated to 56°C. and then kept for 2 hours at 38°C. with the addition of physiological salt solution instead of agar suspension.

There was no expulsion of feces after the first four injections. The introduction into the blood of serum treated with agar caused abundant micturition, followed by rapid expulsion of fifty-five scybala.

The blood drawn 28 minutes after the injection of serum treated with agar showed the first fibrin formation at the end of 18 minutes. Coagulation was completed in 3 minutes more, 21 minutes after drawing the blood. The rabbits died within 2 to 14 hours after the last injection.

Experiments 3 and 4 lead to the conclusion that by keeping normal horse serum for 2 hours at 38°C. in the presence of agar, which has been extracted by centrifugalization and filtration, this serum acquires the power to induce in a normal rabbit certain symptoms of anaphylactic shock; namely, a marked fall in the arterial pressure which persists for a long time, and an abundant expulsion of feces. The serum, previously exposed for 30 minutes to 56°C., does not acquire this property after being treated with agar.

Third Series of Experiments.

Experiment 5.—A rabbit weighing 2,500 gm. received intravenously an injection of 10 cc. of serum treated with agar, which corresponds to 4 cc. per kilo. (Table V.)

TABLE V.

Injection of Filtrate of Serum Treated with Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	8.2	78	11	8.3	78
½	8.8	61	12	8.5	73
1	8.6	61	13	8.6	75
2	8.7	63	14	8.7	64
3	9.0	66	15	8.8	68
4	9.6	56	16	8.9	62
5	9.4	72	17	9.0	70
6	8.7	80	18	9.0	71
7	8.4	80	19	9.0	77
8	8.2	79	20	9.0	69
9	8.4	84	21	9.0	69
10	8.3	78			

With a dose of 4 cc. per kilo the intravenous injection of serum treated with agar induced, in the first place, an increase in the arterial pressure, which reached its maximum (1.4 cm. of mercury) 4 minutes

after the injection. The arterial pressure then returned to its initial maximum level at the 8th minute following the injection, then increased 0.8 cm. of mercury up to the 17th minute after the injection, remaining at this level until the end of the experiment.

The respiration diminished in frequency during the first 4 minutes following the introduction into the blood of serum treated with agar; then it rose to the initial figure where it remained, showing slight variations in both directions (slight accelerations and retardations). There was an expulsion of sixteen scybala immediately after the injection.

The blood drawn from the carotid 12 minutes after the injection showed the first fibrin formation at the end of 7 minutes. Coagulation was completed 15 minutes after the withdrawal of blood.

Experiment 6.—A rabbit weighing 2,300 gm. was injected intravenously with 5 cc. of serum treated with agar, which corresponds to 2.17 cc. per kilo. (Table VI.)

TABLE VI.

Injection of Filtrate of Serum Treated with Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	11.8	123	7	9.7	145
½	12.2	132	8	9.9	121
1	11.8	107	9	9.9	113
2	11.6	108	10	10.0	130
3	11.4	91	11	10.1	105
4	10.5	109	12	10.1	107
5	10.2	117	13	10.1	100
6	9.6	114	14	10.1	95

With a dose of 2.17 cc. per kilo, the intravenous injection of serum treated with agar caused, after a temporary increase in the arterial pressure immediately after the injection, a gradual fall up to the 6th minute after this injection. At this moment the arterial pressure had fallen 2.2 cm. of mercury below the initial level. Then the pressure rose again 0.5 cm. of mercury in 5 minutes, where it remained up to the 20th minute after the injection.

The respiration, which was very rapid before the injection, first

underwent a slight acceleration, followed by a retardation until the 3rd minute after the injection, then an acceleration through the 7th minute, and slight retardations and accelerations. After the injection there was an expulsion of twenty-five scybala.

The blood extracted from the carotid 15 minutes after the injection first showed fibrin formation at the end of 15 minutes. Coagulation was complete 33 minutes after the injection.

Experiment 7.—A rabbit weighing 2,800 gm. received intravenously an injection of 3 cc. of serum treated with agar, or 1.07 cc. per kilo; then 12 minutes later 5 cc. of the same serum treated with agar, or 1.78 cc. per kilo. (Table VII.)

TABLE VII.

Injection of Filtrate of Serum Treated with Agar, 1.07 cc. per Kilo.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	10.0	39	6	8.2	72
½	9.5	47	7	8.1	77
1	9.6	36	8	8.0	86
2	9.5	44	9	7.9	84
3	8.9	67	10	7.9	76
4	8.7	60	11	7.9	78
5	8.3	60			

Injection of Filtrate of Serum Treated with Agar, 1.78 cc. per Kilo.

½	8.1	67	5	7.7	60
1	7.7	49	6	7.7	77
2	7.7	55	7	7.6	68
3	7.7	63	8	7.5	60
4	7.9	65			

The first injection of serum treated with agar, with a dose of 1.07 cc. per kilo, caused within 9 minutes a fall in the arterial pressure corresponding to 2.1 cm. of mercury. During the same time the respiration increased in frequency and reached its maximum, which was more than double the initial number, at the end of 8 minutes.

The second injection of serum treated with agar had hardly any influence on the arterial pressure. Immediately after the injection a slight increase of 0.2 cm. of mercury was produced, then the pres-

sure fell slowly. The respiration, remaining always above the initial frequency, slowed down somewhat, especially 1 or 2 minutes after this new injection. There was no expulsion of feces after the first, and an expulsion of only four scybala after the second injection.

Coagulation, however, was markedly retarded. The blood taken from the carotid 9 minutes after the second injection did not commence to show the first fibrin formation until the end of 20 minutes. Coagulation was not complete until 45 minutes after the extraction of blood.

Experiment 8.—A rabbit weighing 1,450 gm. received an intravenous injection of 5 cc. of the control mixture of serum and physiological salt solution, and 7 minutes later the same quantity of filtrate obtained from the control mixture of physiological salt solution and agar, and finally, 13 minutes after this second injection, 5 cc. of filtrate from serum treated with agar. Each of these injections corresponds to 3.45 cc. per kilo. (Table VIII.)

TABLE VIII.

Injection of Serum and Physiological Salt Solution (Control).

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	8.1	36	3	8.4	39
½	8.9	35	4	8.4	38
1	8.3	38	5	8.3	38
2	8.4	34	6	8.2	41
Injection of Filtrate from Control Mixture of Agar and Physiological Salt Solution.					
½	8.2	39	7	5.5	43
1	8.0	37	8	5.3	40
2	8.5	43	9	5.7	43
3	7.0	39	10	5.8	42
4	6.7	40	11	6.0	42
5	6.3	41	12	6.1	42
6	5.6	40			
Injection of Filtrate of Serum Treated with Agar.					
½	6.9	42	3	6.6	36
1	5.8	53	4	6.4	39
2	6.3	59	5	6.7	38

The arterial pressure rose 0.8 cm. of mercury immediately after the intravenous injection of serum kept for 2 hours at 38°C. in the presence of physiological salt solution. Then it fell 0.6 cm. of mercury and remained a little higher than the initial pressure. It fell until the 8th minute after the introduction into the blood of filtrate from the mixture of physiological salt solution and agar, then it began to rise; the fall amounted to 2.9 cm. of mercury. We encountered here the same phenomenon, though more pronounced, as in Experiment 4. The injection of serum treated with agar, with a dose of 3.45 cc. per kilo, first caused the arterial pressure to rise 0.8 cm. of mercury. This increase was immediately followed by a fall of 1.1 cm. of mercury, then the pressure rose almost to the same level as half a minute after the last injection. The impression obtained is that two opposite actions are produced, and prevent the sharp fall in the arterial pressure which would have been caused by the intravenous injection of serum treated with agar, when the injected dose is 1.07 to 2.17 cc. per kilo (Experiments 7 and 6).

The frequency in respiration was not modified after the intravenous injection either of the control serum or of the filtrate from the mixture of agar and physiological salt solution. It showed a slight and momentary increase after the introduction into the blood of the filtrate of serum treated with agar; then the respiratory rate returned to normal. There was no defecation after the first two injections, but after the last injection there was an expulsion of ten scybala.

Experiment 9.—A rabbit weighing 2,250 gm. received into the marginal vein of the ear 5 cc. of filtrate from the control mixture of physiological salt solution and agar. This dose corresponds to 2.22 cc. per kilo. (Table IX.)

TABLE IX.

Injection of Filtrate from Control Mixture of Physiological Salt Solution and Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	10.6	57	5	4.6	89
½	11.2	60	6	5.0	100
1	10.5	53	7	6.2	98
2	10.1	48	8	7.2	95
3	10.1	56	9	8.0	95
4	3.5	75	10	8.0	80

With this rabbit and the dose employed, the filtrate from the control mixture of physiological salt solution and agar caused, after a slight momentary increase, a rapid fall in the arterial pressure 4 minutes after the injection. The fall amounted to 7.1 cm. of mercury, much more than that obtained with serum treated with agar in this series of experiments. The pressure then rose rapidly, contrary to that observed after the intravenous injection of serum kept for 2 hours at 38°C. in the presence of agar.

The respiration showed, after the 3rd minute following the injection, an acceleration, which reached its maximum 6 minutes after the injection; then the respiration gradually slowed to a slight extent, yet without reaching the initial rate, within 10 minutes after the injection.

There was no expulsion of feces. The blood extracted from the carotid 11 minutes after the injection showed the first fibrin formation at the end of 10 minutes. Coagulation was completed 5 minutes later, 15 minutes after withdrawing the blood.

In the third series of experiments the results were less clear than in the second, because the intravenous injection of filtrate, extracted from the control mixture of agar and physiological salt solution, caused a fall in the arterial pressure more intense, yet more temporary than that caused by appropriate doses of filtrate extracted from serum treated with agar. Besides, this control mixture sometimes caused respiratory acceleration.

Fourth Series of Experiments.

Experiment 10.—A rabbit weighing 2,100 gm. was injected intravenously with 3 cc. of serum treated with agar, 1.43 cc. per kilo; 8 minutes later an injection was given of 6 cc. of the same serum, or 2.86 cc. per kilo. (Table X.)

TABLE X.

Injection of Filtrate of Serum Treated with Agar, 1.43 cc. per Kilo.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	10.4	150	4	10.0	150
½	10.4	150	5	10.0	140
1	10.4	140	6	10.0	160
2	10.3	140	7	10.1	145
3	10.2	150			

Injection of Filtrate of Serum Treated with Agar, 2.86 cc. per Kilo.

½	10.0	140	10	9.4	121
1	9.4	140	11	9.4	123
2	9.5	130	12	9.4	110
3	10.0	140	13	9.4	110
4	9.9	148	14	9.4	102
5	9.8	139	15	9.5	125
6	9.5	130	16	9.3	123
7	9.5	107	17	9.3	105
8	9.4	129	18	9.3	110
9	9.5	133			

The arterial pressure fell 0.4 cm. of mercury 4 minutes after the first injection, and 0.7 cm. of mercury within 16 minutes after the second injection, or 1.1 cm. in all. The respiration did not undergo any modifications after the first injection, but from the 6th minute after the second injection it tended to fall and reached its minimum at the 14th minute after the second injection. After the second injection there was an expulsion of sixteen scybala.

The blood, drawn from the carotid 19 minutes after the second injection, showed the first fibrin formation at the end of 25 minutes. Coagulation was complete 10 minutes later, or 35 minutes after drawing the blood.

Experiment 11.—A rabbit weighing 2,200 gm. received an intravenous injection of 5 cc. of serum treated with agar, which corresponds to 2.27 cc. per kilo. (Table XI.)

TABLE XI.

Injection of Filtrate of Serum Treated with Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	13.3	67	10	10.7	46
½	12.4	68	11	10.5	49
1	12.2	68	12	10.3	44
2	12.1	86	13	10.0	46
3	12.0	102	14	10.0	38
4	11.7	90	15	10.0	39
5	11.9	96	16	10.0	39
6	11.6	74	17	10.0	38
7	12.0	69	18	10.0	39
8	11.7	67	19	10.0	42
9	11.4	52			

The arterial pressure fell 3.3 cm. of mercury in 13 minutes, remaining at this level for at least 6 minutes more. The respiration increased in frequency from the 1st minute, reaching its maximum 3 minutes after the injection. Then the number of respiratory movements gradually decreased, reaching the initial rate 6 or 7 minutes after the injection, and continued falling until the 14th minute after the introduction into the blood of serum treated with agar, showing no further modifications till the end of the experiment. After the injection violent movements of the intestines were observed through the abdominal wall, and there was an expulsion of sixty-nine scybala.

The blood, extracted from the carotid 20 minutes after the injection, showed the first fibrin formation at the end of 20 minutes. Coagulation required 5 more minutes and was complete 25 minutes after drawing.

Experiment 12.—A rabbit weighing 2,550 gm. received an intravenous injection of 5 cc. of filtrate from the control mixture of physiological salt solution and agar, then 14 minutes later 5 cc. of control serum were injected, and finally, 19 minutes after, 5 cc. of serum which had been kept for 30 minutes at 56°C. and then treated with agar. With each injection 1.96 cc. of liquid per kilo were introduced into the blood. (Table XII.)

TABLE XII.

Injection of Filtrate from Control Mixture of Physiological Salt Solution and Agar.

Time after injection.	Arterial pressure.	Respiratory rate.	Time after injection.	Arterial pressure.	Respiratory rate.
min.	*cm. Hg.*	*per min.*	*min.*	*cm. Hg.*	*per min.*
Before.	12.7	110	7	12.6	52
½	12.5	95	8	12.0	54
1	12.2	100	9	11.8	48
2	12.1	105	10	11.6	46
3	11.8	94	11	11.8	40
4	11.8	94	12	12.0	42
5	11.6	87	13	12.6	42
6	11.4	80			

Injection of Serum and Physiological Salt Solution (Control).

½	12.2	46	10	11.6	46
1	12.2	50	11	11.5	44
2	12.2	51	12	11.6	42
3	12.0	40	13	11.6	45
4	12.0	45	14	11.6	45
5	12.4	46	15	11.7	45
6	11.8	48	16	11.8	46
7	11.8	44	17	12.0	47
8	11.5	41	18	12.2	46
9	11.7	47			

Injection of Filtrate of Serum Heated to 56° C. and Treated with Agar.

½	12.1	44	5	12.2	42
1	12.1	42	6	12.3	45
2	12.1	46	7	12.3	45
3	12.1	43	8	12.3	45
4	12.1	45			

The arterial pressure fell 1.3 cm. of mercury 6 minutes after the intravenous injection of filtrate from the mixture of agar and physiological salt solution, and then returned to normal. The injection of control serum produced a fall in the arterial pressure corresponding to 1.1 cm. of mercury in 8 minutes; the pressure then rose slowly, and as late as 18 minutes after the injection it had not reached the initial level. The introduction at this time of serum heated to 56°C.

before being subjected for 2 hours to 38°C. in the presence of agar had no effect on the arterial pressure, which continued to rise slowly.

The respiration decreased in frequency under the influence of the filtrate obtained from the mixture of agar and physiological salt solution until the 11th minute after the injection. The respiratory rate showed no further material modifications, even after the injections—whether of the mixture of serum and physiological salt solution or of the filtrate from the mixture of serum and agar previously subjected to a temperature of 56°C. There was no defecation during the experiment.

Experiments 11 and 12 show a marked effect on the blood pressure by an appropriate dose of serum treated with agar. The fall was more pronounced and more persistent than under the influence of an analogous dose of filtrate from a control mixture of physiological salt solution and agar. In this series of experiments the introduction into the blood of control serum kept for 2 hours at 38°C. in the presence of physiological salt solution produced a slight fall in the arterial pressure, although in the second and third series this injection caused a temporary increase in the pressure.

DISCUSSION.

The experiments show that the intravenous injection of adequate doses of normal horse serum kept for 2 hours at 38°C. in the presence of agar and then freed from this agar through centrifugalization and filtration produces in normal rabbits a marked and prolonged fall in the arterial pressure. To produce this phenomenon to any marked degree, it is necessary to avoid an excess of the serum; for in such a case we should, on the contrary, experience a slight increase in the arterial pressure (Experiment 5). Nor should too small a dose of serum be employed; for in this case we should have only a slight fall in the arterial pressure (Experiments 2 and 10).

A second intravenous injection of serum treated with agar produces but a slight fall in the pressure (Experiments 7 and 10). The adequate dose of serum treated with agar varies in different sera and, to a less extent, in different rabbits.

In order to show clearly the effects of serum treated with agar upon the arterial pressure of normal rabbits, it is necessary to inject into

the rabbits increasing doses of serum: 1.00, 1.25, 1.50, 1.75, and 2.00 cc. per kilo, and, according to the results obtained, smaller or larger quantities. Each dose must be injected into two or three rabbits. When the proper dose per kilo is thus established, we proceed with tests using an equal dose of each control liquid in two or three normal rabbits. Likewise we should employ in the same animal only one kind of liquid (serum treated with agar or one of the control mixtures). Unfortunately, we did not have at our disposal the number of animals necessary for the technique outlined above.

We wish to emphasize the fact that it is preferable to employ, as in the experiments with guinea pigs, homologous serum; that is, rabbit serum.

Our results are supported by those of Bordet and Zunz and of other investigators who have studied the action of serum kept at 38°C. in the presence of agar and then separated from the latter material.

In normal rabbits this serum produces the same modifications in the arterial pressure as those produced by intravenous injections of horse serum in sero-anaphylactized rabbits. Before treatment with agar sometimes this is not the case and even a slight fall in the pressure may be produced. The filtrate from the control mixture of agar and physiological salt solution at times produces a pronounced fall of the arterial pressure, but this fall differs from that caused by serum treated with agar, since the pressure returns relatively rapidly to its initial level in the first case, whereas in the second the pressure remains for a long time at quite a low level.

The modifications in respiration are not very pronounced after the intravenous injection of serum treated with agar. The respiratory rate, nevertheless, increases to a certain degree, four times out of twelve, after this injection (Experiments 4, 7, 8, and 11).

This occurred once out of four times after the injection of filtrate from the mixture of agar and physiological salt solution (Experiment 9), and once out of five times after the injection of control serum kept for 2 hours at 38°C. in the presence of physiological salt solution (Experiment 4).

Serum treated with agar has a marked effect on the intestinal movements. Expulsion of feces never occurred under the influence of the control mixtures.

Coagulation in the carotid blood was at times very much retarded (Experiment 7) under the influence of intravenous injection of serum treated with agar. At other times the phenomenon was less pronounced. With the same amount of the same kind of serum, the intensity corresponded to the degree of the fall in the arterial pressure. There was little or no retardation in coagulation, and the pressure showed little or no fall. A marked relation seemed to exist between these two phenomena.

The intravenous injection of filtrate from the mixture of agar and physiological salt solution did not cause any obvious modifications in the coagulation of the carotid blood, even though the arterial pressure underwent a pronounced fall but one of short duration (Experiment 9). In this respect, the effects of this injection were distinctly different from those due to the introduction of serum treated with agar into the circulatory system of normal rabbits.

Horse serum exposed to a temperature of 56°C. for 30 minutes and treated with agar does not cause, in normal rabbits, a pronounced and lasting fall in the arterial pressure and the other phenomena produced under the influence of the intravenous injection of the normal serum kept for 2 hours at 38°C. in the presence of agar.

SUMMARY.

The intravenous injection of horse serum, kept for 2 hours at 38°C. in the presence of one-fifth of its volume of a suspension of 0.5 per cent agar in physiological salt solution and then separated from the agar by centrifugalization and filtration, produces in normal rabbits in adequate doses a considerable and prolonged fall in the blood pressure, expulsion of feces, a diminished coagulability in the carotid blood, and at times accelerated respiration; that is, the various symptoms observed after the intravenous injection of horse serum in a sero-anaphylactized rabbit.

Horse serum previously kept for 30 minutes at 56°C. and then treated with agar in the manner described above, when injected intravenously into a normal rabbit will have no more effect on the arterial pressure, on the intestinal movement, on the respiratory rate, or the coagulation of arterial blood than the introduction of horse serum into the veins of a normal rabbit.

AN EXPERIMENTAL STUDY OF CIRCUMSCRIBED DILATION OF AN ARTERY IMMEDIATELY DISTAL TO A PARTIALLY OCCLUDING BAND, AND ITS BEARING ON THE DILATION OF THE SUBCLAVIAN ARTERY OBSERVED IN CERTAIN CASES OF CERVICAL RIB.*

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Plates 16 to 19.

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No one, since Deitmar,[1] has attempted to collate the cases of dilation of the subclavian artery associated with cervical rib. Deitmar cites five cases (Adams, Coote, Poland, Baum, and von Heinecke), including one (von Heinecke's) which I have tabulated as doubtful. Streissler's review[2] is perhaps the fullest in the literature on the subject of cervical rib. Although it appeared less than 3 years ago no addition is made by this author to Deitmar's list.

From a careful study, in the original, of the reports of 716 cases of cervical rib I find that aneurysm or dilation of the subclavian artery was noted in 27 or more of them, including six (Mayo, Murphy, Russel, von Heinecke, Galloway, and Seymour) in which the surgeon believed that the vessel was abnormally large, and two (Karg and Halsted) in which the aneurysm appeared promptly after removal of the supernumerary rib. There may be numerous other instances of dilation of the subclavian associated with cervical rib—cases in which the amount of arterial expansion could not be determined in the lack of a standard of comparison.

The Dilation of the Subclavian Artery Is Distal to the Line of Constriction Made by the Rib and the Scalenus Anticus Muscle.

As to the cause of these aneurysms there is much conjecture. The comment has frequently been made that their occurrence would be

* It is possible in the limits of the permissible space to present the results of the work of Dr. Reid and myself in merest outline. A full account will probably appear next year in the *Reports of the Johns Hopkins Hospital.*

[1] Deitmar, J., Inaug. Diss., Erlangen, 1907.

[2] Streissler, E., *Ergebn. Chir. u. Orthop.*, 1913, v, 280.

comprehensible if they appeared on the proximal instead of the peripheral side of the compression. No one has remarked that dilation central to the site of pressure might be even more difficult to comprehend.

The suggestions which have been offered in the effort to explain the phenomenon are as follows: (1) weakening of the wall of the artery from erosion or other trauma; (2) variable or intermittent pulse pressure; (3) vasomotor and vasa vasorum disturbances.

In 1906 Dr. E. H. Richardson and I made the observation that after partial occlusion of the thoracic aorta the maximum pressure may be permanently lowered and the minimum pressure actually increased distal to the constricting band of metal. This discovery was verified by Dr. Dawson on one of my dogs (No. 96).

Dog. 96. Partial Occlusion of the Thoracic Aorta.

Operation, May 22, 1906. Killed 7 months later.

	Maximum pressure.	Mean pressure.	Minimum pressure.	Pulse pressure.
Femoral	116	93	88	28
Carotid	160	113	83	77

The dilation of the artery observed in arteriovenous fistula might, I thought, have a bearing on the interpretation of the aneurysms in cases of cervical rib. "May not both phenomena," I asked myself, "be due to degenerative changes in the arterial wall consequent upon lowered pressure?"

Inasmuch as dilation of the subclavian artery has, relatively to the number of cases of cervical rib, so seldom been observed, it seemed that if it were due merely to the lowered pulse pressure, then only a very definite absolute or relative amount of reduction of the systolic pressure would suffice to produce it. It was realized, also, that even if the amount of reduction necessary to accomplish the desired result could be determined it could not be constantly maintained, inasmuch as the peripheral resistance becomes, in great measure, rapidly restored.

For a number of years, in the course of various experiments in partial occlusion of the arteries, I had somewhat in view the chance of there being produced beyond the point of constriction a dilation of

the artery analogous to that which had been observed in cases of cervical rib.

The Degree of Constriction and the Period of Time Required for the Production of the Dilation.

2 years ago when, after many trials, I had altogether despaired of having the hope realized, I was startled, on examining the abdomen of a dog whose aorta had been constricted for 5 months and 20 days, to see that each of the branches of trifurcation was dilated almost to the size of the main aortic trunk. About to leave town for the summer, I communicated the finding to Dr. Reid, asking him promptly to repeat the experiment, as precisely as possible. In the autumn we expectantly laparotomized three dogs upon which Dr. Reid had operated in the early summer and were disappointed to find that no change had taken place in the size of the aorta or its three terminal branches. Confident that there could have been no error in the original observation, I constricted the abdominal aorta, to various degrees, just above the trifurcation in twenty dogs and, at intervals, in the course of the winter, explored and reexplored the abdominal cavities, but with negative result, at least as concerned dilation.

Finally, on opening the abdomen of our last dog (No. 7) we found the dilation which we sought. The result is depicted in Figs. 1 to 4. The occlusion of the aorta in this case made 6 months and 19 days previously was almost complete, just as it had been in the one successful case of the foregoing year—I might say, of all the foregone years.

The pressure exercised by the band in this instance had been sufficient to lessen greatly, if not to obliterate, for a few moments at least, the palpable thrill produced by the constriction, but not enough completely to shut off the palpable pulse. With this observation as a fresh incentive, Dr. Reid and I have continued the experiments during the current academic year with encouraging results.

If the occlusion must be so nearly complete in order to effect a well pronounced dilation, it will explain not only the difficulty we have had in producing it in dogs, but also the fact that it has been observed so seldom in the human subject from compression of the subclavian

artery by a cervical rib. For when in dogs the aortic pulse is occluded beyond the stage of palpable thrill the lumen is in danger of becoming obliterated, as by the formation of a cylindrical fibrous cord beneath the band, and thus cancelling the experiment; and in the cervical rib cases we may assume argumentatively that the subclavian artery compressed to the stage sufficient to produce an aneurysm might become thickened by sclerotic changes in the time required for the pronounced manifestation of a dilation. Thus, a moderate dilation, present for a brief period prior to occlusion of the subclavian, might be overlooked.

In dogs, a number of months must apparently elapse after the application of the band before a dilation in striking degree can occur. In the two cases (No. 7, 1913, and No. 7, 1914) to which reference has been made, 5 months and 20 days, and 6 months and 19 days, respectively, had elapsed. In the second of these, a dilation of the middle branch of the trifurcation of 2 mm., found at the expiration of 60 days, had increased to 4 mm. in the course of the following $4\frac{1}{2}$ months (Figs. 1 to 4). Other instances of like dilation are accurately represented in Figs. 5 to 11. The amount of constriction made in two of the illustrated cases (Dogs 3 and 15, Series II) is indicated by the concentric circles of Figs. 8 and 11.

We have found and abstracted reports of 716 instances of cervical rib, in great part from the original.

Clinical cases		525
Autopsies		91
Museum specimens		100
Total		716
		per cent
Cases with nerve symptoms alone	235	65.3
Cases with nerve and vascular symptoms	106	29.4
Cases with vascular symptoms alone	19	5.3
Total	360	

525 were clinical cases; 91, autopsy findings; and 100, museum specimens. 360 presented symptoms of pressure. Of these, 235 had nerve symptoms alone; 106, nerve and vascular; and 19, only vascular symptoms. Accordingly we have reports of 125 cases of cervical rib

in which vascular symptoms were noted. In 27 of these (21.6 per cent) an enlargement, fusiform, aneurysmal, or cylindrical, was observed, and, of these, in the majority, the disturbance of circulation was severe, 6 cases having gangrene of fingers on the affected side (Table I).

Of the thirty dogs with aortic constriction upon which this report is based there was pronounced dilation, for a short distance, of the vessels below the band in seven, or 23.3 per cent.

It is interesting to note the correspondence in the human (21.6) and canine (23.3) percentages. We must not, however, overvalue the result of this haphazard sort of comparison, for, as regards the human cases we have depended upon the impressions of surgeons, who, having no standards of comparison, having made no measurements, and not always being particularly concerned about the arterial feature of the case, may have overlooked or overestimated variations from the norm; and, as regards the thirty dogs, we observed, in addition to the seven designated as major dilations, ten minor ones. The seventeen dilations of all grades represent 56.6 per cent of the thirty dogs. The percentage is even greater if we include in our calculation only the dogs of the past year; and, for another year of experimentation, would probably be greater still.

We may, I think, conclude that the dilation of an artery produced experimentally is not due to any of the three factors proposed as causal for the aneurysm in cases of cervical rib.

1. Vasomotor Paralysis.—(*a*) The vasomotor nerves and the vasa vasorum are destroyed by the moderately constricting and totally occluding bands quite as surely as by those which, occluding almost totally, alone have produced the dilation. (*b*) Only a portion of the circumference of the subclavian artery is exposed to the pressure of the cervical rib and the scalenus anticus muscle, and hence only a fraction of the vasomotor nerves or vasa vasorum could be pressed upon.

2. Trauma.—(*a*) Usually, the dilation is fusiform and (*b*) distal to the rib. (*c*) Trauma is excluded as a factor in the experimental dilations.

3. Inconstant Blood Pressure.—(*a*) Patients suffering from the pressure-pain of cervical rib rarely make wide excursion-movements of the arm. (*b*) The degree of occlusion is constant in the experimentally constricted vessel.

When an arterial trunk is ligated it becomes occluded to the first proximal and first distal branches by a process of cell proliferation which ultimately reduces the artery to a fibrous strand.

Is There a Fall in Blood Pressure in the Dead Arterial Pocket Which May Anticipate and Possibly Be a Causative Factor in the Obliteration of This Portion of an Artery?

From observations which I have made on man and dogs I am quite sure that there may be a remarkable fall in blood pressure in what I have termed the "dead arterial pocket," while there is still little if any sign of diminution in the caliber of this portion of the vessel. For example, the right common carotid artery was ligated by the writer in a case of aneurysm of the external carotid. About 3 months later, in the course of an operation for the excision of the uncured aneurysm, the internal carotid, dead-pocketed between the circle of Willis and the carotid ventricle, was freely exposed for a considerable distance. It had lost its cylindrical form, being flat and tape-like, and, although evidently possessing a considerable lumen, seemed to be empty. When pricked, a few drops of blood oozed without pulse from the little cut. The artery was then resected between two ligatures. Its wall was thickened on one side (Fig. 12) but the lumenwas still perhaps three times that of a radial artery. Similar observations I have made twice on the external iliac artery of the dog after occlusion of this vessel at its origin from the aorta. In the approximately dead pocket between the aorta and the origin of the circumflex iliac and common trunk of the epigastric and obturator arteries the blood pressure must have been almost nil, because from a little slit made in the apparently normal arterial wall of the relatively empty external iliac artery the blood escaped very slowly in a tiny, almost pulseless jet about 1 cm. high; whereas, from the femoral artery, below the profunda, the blood spurted normally from a similar knife-prick.

Hence in an artery doomed to obliteration, it would seem that the blood pressure may be lowered before the occlusion process sets in—the lowered pressure being, perhaps, the immediate factor leading to the obliteration.

Consideration of the Cause of the Dilation.

Can these observations have any bearing upon the explanation of the dilation of the aorta above its trifurcation, of its triad branches,

and of the carotid, which we have occasionally observed in the dog distal to the partially occluding band?

The mechanical engineer knows the effect upon pressures of constricting a rigid tube through which water is being forced at a given pressure. At the site of the constriction, of the Venturi meter, the pressure is diminished and the velocity increased, whereas immediately beyond the constriction both the normal pressure and velocity are restored. This is not, however, true of the constricted arterial tube. Beyond the band the systolic pressure may be lowered, the diastolic pressure increased, and the pulse pressure greatly reduced for many months at least.

If the constriction of the aorta is very slight the effect upon the blood pressures is usually transient, the normal pressures being reestablished within a few hours or, indeed, minutes. But if the artery is constricted to the point of almost total occlusion the pulse pressure below the band, for a time almost nil, may remain lowered and the diastolic pressure, relatively or even actually, be increased. The blood stream in this case, passing with greater velocity and less pressure through the band prevents the obliteration of the artery to the nearest branch, the pocket being not a dead one as it is in the case of total obliteration. The blood in this pocket beyond the constriction streams in whirlpools, somewhat as in the vein and, also, as in the artery in arteriovenous fistula; the thrill, not palpable at first, later may be perceived with the finger; and the bruit, always audible with the stethoscope, becomes louder as the peripheral arterial resistance increases.

To these factors, then,—to the abnormal play of the blood in the relatively, as distinguished from the absolutely dead pocket and to the absence of normal pulse pressure, essential probably to the maintenance of the integrity of the arterial wall, we may have to look for the solution of our problem.

It is not, however, denied that the paralysis of the vasomotor nerves and the occlusion of the vasa vasorum may possibly play some part in the manifestation. The dilations produced experimentally, like those observed in cases of cervical rib, are, as I have said, circumscribed. We had conjectured that the delimitation of the dilation might be influenced or determined by the location of the branches of the affected artery beyond the constriction. In one instance,

however, we observed, just distal to a partially occluding band applied to the carotid low in the neck of a dog, a circumscribed dilation of this vessel, the branches of which had been tied and divided. In this case there was a long stretch of debranched and patulous carotid between its dilated portion and the base of the skull.

Do Intimal Surfaces Brought Gently in Contact Unite, and What Is the Process by Which Obliteration of an Artery Takes Place after Occlusion by Band or Ligature?

The intimal surfaces of arteries brought intact in apposition whether by ligature or by band never, in my experience, have united. This statement will be received sceptically, for it is at variance with the quite universally accepted view, that uncrushed intimal surfaces if brought gently in contact adhere and thus occlude the artery. In the ligation of the larger arteries we have been taught to use heavy ligatures, two or three abreast, and in tightening them to employ only enough force to bring the intimal surfaces in contact, a force not sufficient to rupture or injure the intimal coat. The gross and microscopic findings in the sections of arteries ligated in this manner have been repeatedly portrayed, but the illustrations submitted as proof are not convincing.

It is my opinion that the pressure necessary to bring about the complete closure of the aorta causes atrophy of the arterial wall under the band, and that union of the apposed surfaces thus deprived of their blood supply does not occur. To accord with this view, how is to be explained the formation of the fibrous cylinder which we occasionally find encompassed by the band, and how the probable error of other investigators who believe that intimal surfaces brought gently in contact by broad ligatures unite primarily and thus interrupt the blood stream?

The process of occlusion is, I believe, somewhat as follows: The death of the arterial wall having been brought about by the pressure of the band, a gradual substitution or organization of the necrotic tissue takes place, the new blood vessels penetrating it from both ends. The absorption of the lifeless wall proceeds coordinately with its vascularization or organization.

If the band has been rolled so tightly as to occlude the lumen, the arterial wall is deeply puckered or plicated. If after a month or two the aorta is cut open at this point, the folds of the arterial wall may still be seen. They will not be adherent to each other, but atrophied possibly to the thinness of writing or tissue paper. The attenuated wall can still be completely unfolded. In some cases it is found to be abnormally thick and a very fine lumen to have been established (Fig. 13). In this event there are no folds, the thick wall consisting altogether of new tissue, and the old wall with its plication having been absorbed. Consequent upon the atrophy of the arterial wall the tension under the band is eliminated, capillaries sprout into the necrotic part, which thus becomes replaced by new connective tissue. A tiny blood stream may make its way under the band, and an endothelial lining for the new wall develop.

More often we find in the completely or almost completely occluded cases that a solid, fibrous, cylindrical cord completely fills the space within the band and replaces the original wall of the artery.

Although we are unable to share the opinion of other investigators that the uninjured intimal surfaces of large arteries adhere to each other when brought in contact, the advice to bring broad surfaces in apposition by several contiguous, coarse ligatures is good. We have found that the finer the ligature the quicker it cuts through the artery, very fine silk cutting through in a day or two. Narrow tape, constricting the artery in spiral or cuff-like form, has about the same effect upon the arterial wall as the metal band. Under three or four coarse ligatures, drawn tightly enough to occlude the lumen but not so tightly as to injure the intima, the arterial wall would behave presumably as it does under the tape or band, and occlusion take place in the described manner. I should think, however, that it would be impossible in a given case to be sure that each one of three ligatures, for example, had occluded the artery without injury to the intima. Even with the metal band, which can be rolled with great precision and in perfect, cylindrical form, it is not possible to say that occlusion has no more than just been attained, although we can be certain that the intima has suffered no trauma. But with the ligature pressure cannot be exerted in such fine and exact gradations; there is also the complication of the knot and its particular pressure.

The surgeon's conceptions as to the finer processes concerned in the occlusion of arteries after ligature are based largely upon studies undertaken before the days of perfected asepsis, in the days when thrombus formation almost invariably complicated the picture.

SUMMARY.

1. A partially occluded artery may dilate distal to the site of constriction.

2. The dilation is circumscribed.

3. When the constriction has been either slight in amount or complete, dilation has not been observed.

4. The dilation was greatest when the lumen of the artery (the aorta) was reduced to one-third or perhaps one-fourth of its original size (Figs. 8 and 11).

5. Dilation or aneurysm of the subclavian artery has been observed twenty-seven or more times in cases of cervical rib.

6. The dilation of the subclavian is circumscribed, is distal to the point of constriction, and strikingly resembles the dilation which we have produced experimentally.

7. The genesis of the experimental dilation and of the subclavian dilation occurring with cervical rib is probably the same.

8. When the lumen of the aorta is considerably constricted the systolic pressure may be permanently so lowered and the diastolic pressure so increased that the pulse pressure is greatly diminished.[3]

9. The experimentally produced dilations and the aneurysms of the subclavian artery in cases of cervical rib are probably not due to vasomotor paralysis, trauma, or sudden variations in blood pressure.

10. The abnormal, whirlpool-like play of the blood in the relatively dead pocket just below the site of the constriction, and the lowered pulse pressure may be the chief factors concerned in the production of the dilations.

11. Intimal surfaces brought, however gently, in contact by bands or ligatures do not, in our experience, unite by first intention, for

[3] See Reid, M. R., *J. Exp. Med.*, 1916, xxiv, 287.

the force necessary to occlude the artery is sufficient to cause necrosis of the arterial wall.

12. Bands, rolled ever so tightly, do not rupture the intima.

13. The death of the arterial wall having been brought about by the pressure of the band, a gradual substitution of the necrotic tissue takes place, the new vessels penetrating it from both ends. It is, I believe, in this manner that an artery becomes occluded, and it is thus that a fibrous cord forms within the constricting band.

TABLE I.

Cases of Dilation of the Subclavian Artery with Cervical Rib.

Surgeon.	Publication.	Note on dilation of subclavian artery.	Remarks.
Mayo, H.	*London Med. and Phys. J.*, 1831, N. S., xi, 40.	Right subclavian artery flattened. Unusual width.	
1. Adams, R.	*Dublin J. Med. Sc.*, 1836, xvi, 494.	Ovoid dilation of 3rd portion, left subclavian, about size of pigeon's egg. Postmortem confirmation.	Radial pulses equal.
2. Warren, J. M.	*Am. J. Med. Sc.*, 1849, xvii, 13.	Aneurysmal tumor, left subclavian, about size of pigeon's egg.	Dec. 24, 1847. Ligation of 3rd portion. Ligature came away on 96th day.
3. Coote, H.	Lancet, 1861, i, 360.	Pulsating swelling of left subclavian, about size of walnut.	Part of cervical rib resected. Radial pulse hardly palpable before operation.
4. Poland, A.	*Med.-Chir. Tr.*, 1869, lii, 277.	Right subclavian—"Circumscribed dilatation of entire circumference."	"Digital compression on cardiac side of tumor for 96 hours." Cure of aneurysm.
5. Baum, W.	*Deutsch. Chir.*, 1880, Liefg. 34, 25. (Fischer, G.)	"Left subclavian aneurysm."	Ice, elevation of arm, compression. Aneurysm cured. Pulse absent in radial and ulnar, but present in brachial.
6. Gould, A. P.	*Tr. Clin. Soc.*, 1884, xvii, 95.	"Right subclavian artery projected even beyond clavicle as tense pulsating cylinder."	No operation. Progressive obliterative arteritis. Dilation uninfluenced.
7. Morris, W. C.	*Lancet*, 1885, ii, 152.	Fusiform aneurysm of right subclavian, ¾ in. in width.	Operation. Central ligation of 3rd portion. Radial pulse before operation weak—absent after operation—but reappeared in 1 month or less.

* Karg.	*Beitr. klin. Chir.*, 1895, xiv, 215. (Ehrich, E.)	Aneurysm of left subclavian appeared after removal of the cervical rib.	Artery compressed between 2 cervical ribs. Both ribs removed. Aneurysm cured by compression.
8. Stifler.	*Münch. med. Woch.*, 1896, xliii, 544.	Pulsating tumor of left subclavian. Sudden appearance.	Blood pressure, left 120; right 140. No operation.
9. Cohen, J.	Inaug. Diss., Göttingen, 1898.	"Left subclavian aneurysm size of plover's egg."†	"Weaker radial pulse." Fusiform dilation.—"7 cm. long and 1½ fingers thick." Aneurysm excised.
10. Grisson.	*Fortschr. Geb. Röntgenstrahlen*, 1898–99, ii, 103.	Right subclavian, "pulsating cord, thickness of little finger."	Pulseless axillary, brachial (hard cord), and radial. Aneurysm cured by suspension, Priesnitz bandage, etc.
11. Winkler, C.	*Nederl. Tyschr. Geneesk.*, 1904, xl, 589; *Münch. med. Woch.*, 1904, li, 2111.	Left subclavian—"Locally aneurysmally dilated."	Cyanosis of left hand.
12. Murphy, J. B.	*Ann. Surg.*, 1905, xli, 399.	Left subclavian "flattened and circular."	Brachial and radial pulses normal with arm extended. Operation. Dilation questioned by author in 1905, but called abnormal dilation in 1906.
? Murphy, J. B.	*Surg. Gynec. and Obst.*, 1906, iii, 514.	Right subclavian flattened out on surface of rib.	(Circulation?) Dilation questioned by author. (Exophthalmic goiter.)

TABLE I.—*Continued.*

Surgeon.	Publication.	Note on dilation of subclavian artery.	Remarks.
13. Conner, L.	*Med. Rec.*, 1906, lxix, 775.	Left subclavian—"Broad pulsating mass suggestive of aneurysm."	No note as to circulation or treatment.
14. Keen, W. W.	*Am. J. Med. Sc.*, 1907, cxxxiii, 173.	Left subclavian dilated to nearly twice width of artery.	Gangrene. Operation. Cylindrical dilation to clavicle or beyond.
? Russel, C. K.	*Med. Rec.*, 1907, lxxi, 253.	*Autopsy Note.*—Right subclavian proximal to thyroid axis, large and soft; distal to this, contracted and cord-like. Left subclavian enlarged.	Gangrene. Amputation of index finger, and later of arm. Patient would not consent to operation on rib. Suicide.
? von Heinecke, W. H.	Inaug. Diss., Erlangen, 1907, 40. (Deitmar.)	Left subclavian "surprisingly broad." (Operative note.)	Left radial pulse weaker than right, not improved by resection of rib.
* Halsted, W. S.	Unpublished (1909).	Dilation of right subclavian developed after removal of cervical rib.	The aneurysm, size of plover's egg, seemed to involve the artery central to the site of constriction.
15. Hamann, C. A.	*Cleveland Med. J.*, 1910, ix, 453.	Right subclavian beyond rib enlarged and filled with clot.	Gangrene of fingers. Bursa ¾ in. in diameter between artery and rib. Resection of part of the rib.
16. Pringle, J. H.	*Edinburgh Med. J.*, 1911, vii, 253.	Left subclavian—fusiform aneurysm distal to rib—sacculated at its proximal part under outer edge of scalenus.	Aneurysm twice needled (before discovery of rib). Finally opened and rib removed. Radial pulse "feebler;" became fair after operation.

17. Miller, J. L.	*Am. J. Med. Sc.*, 1911, cxlii, 811.	Side not stated. Slight dilation above the notch in cervical rib.	Cyanosis of hand.
Galloway, J.	*Proc. Roy. Soc. Med.*, 1911–12, v, 112.	Left subclavian—"pulsating tumor."	Unoperated.
? Seymour, N. G.	*Am. J. Med. Sc.*, 1913. cxlvi, 396.	Right subclavian—small pulsating tumor, "simulating" aneurysm.	Female, 16 years. Radial pulse unaffected.
18. Law, A. A.	*J.-Lancet*, 1914, xxxiv, 333.	"Right subclavian dilated distal to rib." "Left subclavian flattened."	Gangrene of fingers. Resection of rib on both sides.
19. Potter, C.	*J. Missouri Med. Assn.*, 1914–15, xi, 74.	Left subclavian—"Pouch-like dilatation of the wall of the artery over groove in cervical rib."	Male, 17 years. Ulcers and gangrene of thumb and ring finger. Brachial and radial pulses absent.

In the unnumbered cases the dilation is questionable. In the cases marked with an asterisk the aneurysm appeared after removal of the rib.

† Possibly due to fracture of the clavicle.

EXPLANATION OF PLATES.

PLATE 16.

DOG 7, SERIES I. THE FIGURES ARE ACTUAL SIZE.

FIG. 1. Aorta before the band was applied.

FIG. 2. Immediately after application of the band.

FIG. 3. 2 months thereafter.

FIG. 4. 6 months and 19 days thereafter.

PLATE 17.

DOG 3, SERIES II. THE FIGURES ARE ACTUAL SIZE.

FIG. 5. Aorta before the band was applied.

FIG. 6. Immediately after application of the band.

FIG. 7. 97 days thereafter.

FIG. 8. 9 months thereafter. The outer of the two concentric circles indicates the circumference of the aorta at the site of the band before its application; the inner circle, the precise lumen of the aorta under the band when the dog was killed.

PLATE 18.

DOG 15, SERIES II. THE FIGURES ARE ACTUAL SIZE.

FIG. 9. Aorta before the band was applied.

FIG. 10. Immediately after application of the band.

FIG. 11. 160 days thereafter. The outer of the two concentric circles indicates the circumference of the aorta at the site of the band before its application; the inner circle, the precise lumen of the aorta under the band when the dog was killed.

PLATE 19.

SECTION OF HUMAN INTERNAL CAROTID.

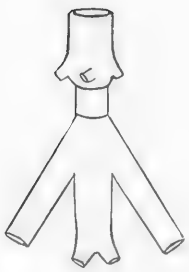
FIG. 12. Section of human internal carotid, showing the process of obliteration after ligation of the common carotid. When removed at a subsequent operation this artery was found pulseless and almost empty notwithstanding the size of its lumen. × 25.

DOG 9, SERIES II. THE FIGURE IS ACTUAL SIZE.

FIG. 13. The band was rolled so tight as to shut off the palpable thrill but not the pulse. When killed after 6 months and 8 days the lumen at the lower edge of the band was 1.2 mm. in diameter. A new wall had been formed under the band, thick throughout, but particularly so at the lower and upper borders of the band. It is probable that, in this case, a fibrous cord ultimately would have formed.

1

2



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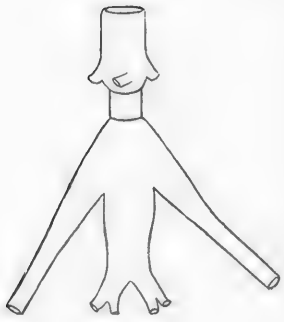
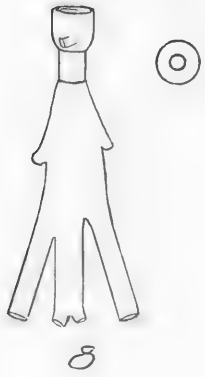


4

(Halsted: Circumscribed Dilation of an Artery.)



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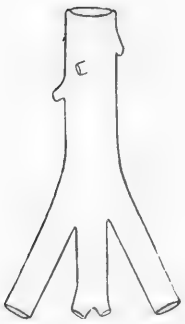

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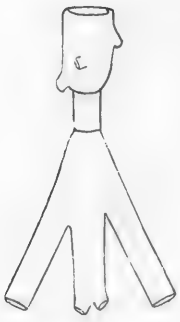

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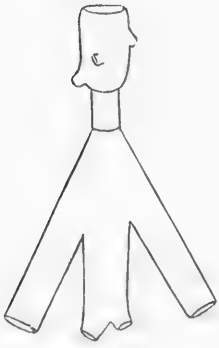

11

(Halsted: Circumscribed Dilation of an Artery.)

12

13

(Halsted: Circumscribed Dilation of an Artery.)

PARTIAL OCCLUSION OF THE AORTA WITH THE METALLIC BAND. OBSERVATIONS ON BLOOD PRESSURES AND CHANGES IN THE ARTERIAL WALLS.*

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PLATES 20 TO 26.

(Received for publication, June 27, 1916.)

In all except one of the aortic experiments of Dr. Halsted and myself the constricting aluminum band was applied to the abdominal aorta below its inferior mesenteric branch. At the time of our final observations on these animals records were made of the blood pressures in the femoral and carotid arteries. Obviously, in order to draw any conclusions as to the effect of the band on the blood pressure below the site of the constriction, the normal relation between the pressures in these two vessels must be known.

In a series of experiments performed by Dr. Dawson on dogs, it was learned that the pulse pressure in the femoral artery is normally about twice as high as in the carotid. The femoral systolic pressure is higher and the diastolic pressure lower than the corresponding pressures in the carotid artery (Fig. 1).

After partial occlusion of the aorta the systolic pressure in the femoral is markedly lowered. This lowering of the systolic pressure is due mainly to a fall in the pulse pressure, for the diastolic pressure remains almost stationary, or may be actually increased. In the cases of most marked dilation the femoral pulse pressure was only about one-half the carotid pulse pressure, while the femoral diastolic was actually greater than the carotid diastolic pressure (Fig. 2).

*This communication is to supplement Dr. Halsted's paper on Circumscribed Dilation of an Artery Immediately Distal to a Partially Occluding Band, *J. Exp. Med.*, 1916, xxiv, 271.

During the 1st hour after the application of a moderately tight band the femoral pressures undergo marked changes. At first the systolic and diastolic pressures are both lowered. In a few minutes the diastolic pressure may become even greater than before the application of the band, while the systolic is still subnormal (Fig. 3).

After complete occlusion of the aorta the normal blood pressure relation between the femoral and carotid arteries may, ultimately, in some instances, be reestablished.

Gross Effect of the Band on the Vessel Wall.—In some cases in which the band has been loosely applied, only slight gross alteration in the wall of the vessel under the band is found, even after 6 months. On removal of the band the plications of the wall can be unfolded, and the intima presents a normal looking, smooth surface (Fig. 4).

In the majority of cases there occurs an atrophy or necrosis of the vessel wall included in the band. In some cases the band had made its way through the atrophic wall into the lumen of the vessel (Figs. 5, 6, and 7); in these a new wall had formed outside the band. In no instance was there leakage of blood. On splitting open the artery for examination the band is seen, more or less distinctly, shimmering through the atrophic arterial wall. This wall may be attenuated to a veil-like thinness or, as described, may, in places, have entirely disappeared, disclosing portions of the band. The upper edge of the band, at the posterior surface of the arterial wall, is almost invariably the first part to be exposed (Figs. 5 and 6).

Histologic Changes.—For a short distance below the site of the band there is usually a definite atrophy of the elastic and muscular tissues. In Case 7, Series I, (Fig. 8, *c*), there was almost a complete break in the elastic tissue below the edge of the band. The connective tissue throughout the wall of the artery seemed to be little affected in amount in the dilated portion of the vessel.

At the site of the band the new wall that forms over it is composed mainly of fibrous tissue (Figs. 7 and 9). Thus far, none of our cases have shown regeneration of the elastic tissue in this new wall.

In the fibrous cord (Figs. 10, 11, and 12) which occasionally forms under the tightly rolled band no remains of the vessel wall have been found. We think it probable that the original arterial wall undergoes complete atrophy and absorption in these cases, and that

the cylindrical cord found under the band consists of new tissue which, growing in from above and below, replaces the old. This cylindrical fibrous cord may be highly vascularized (Fig. 12). We have found no evidence of union between the apposed intimal surfaces.

EXPLANATION OF PLATES.

PLATE 20.

FIG. 1. The normal relation between the systolic and diastolic end pressures in the femoral and carotid arteries of dogs.

FIG. 2. Systolic and diastolic end pressures in Dog 7, Series I, 6 months and 19 days after the band was applied. Good dilation.

FIG. 3. Systolic and diastolic end pressures in the femoral artery during the first 35 minutes after a tight band was placed around the aorta.

PLATE 21.

FIG. 4. Dog 9, Series I. The band was rolled to the early stage of palpable thrill. The band in 6 months has produced only slight thinning of the vessel wall.

FIG. 5. Dog 7, Series II. Marked constriction. The thrill was not obliterated. The band had, in 6 months, cut entirely through at its upper edge, posteriorly. At the lower edge of the band there is still a remnant of the atrophied wall (compare Fig. 7).

FIG. 6. Dog 0, Series II. The band was tightly rolled but not totally occluding. At autopsy, 5½ months later, the lumen under the band was about 3 mm. in diameter. The aortic wall was so thin that the band shimmered through it everywhere, and had cut through at several places along the upper and lower edges. A new arterial wall, almost complete, had formed outside the band.

PLATE 22.

FIG. 7. Dog 7, Series II. Longitudinal section through the arterial walls, old and new (compare Fig. 5). Weigert's elastic tissue stain. *a* is the site of the band; *b*, elastic tissue of the aortic wall proximal to the band; *c*, elastic tissue distal to the band; *e*, the new wall which had formed outside the band where it had cut through, posteriorly. Between *a* and the lumen of the vessel the original wall is greatly thickened.

FIG. 8. Dog 7, Series I. The band was tightened until the thrill disappeared; the pulse was not obliterated. Killed after 6 months and 19 days. Longitudinal section through the arterial wall at the site of the band. Weigert's elastic tissue stain. In this case there was marked dilation below the band. *a* is the

site of the band; *b*, the aortic wall proximal to the band; *c*, the aortic wall distal to the band, showing definite atrophy and a break in the elastic tissue; *e*, the new wall that had formed outside the band; *x* to *x*, the segment of the vessel wall that had been included by the band. In this segment there is marked atrophy of the elastic tissue, particularly at the edges of the band.

PLATE 23.

FIG. 9. Dog 17, Series I. The band was tightened to the vanishing side of the thrill stage. Longitudinal section through the arterial wall at the site of the band. Weigert's elastic tissue stain. Under the band the elastic tissue is rarified. Below the band, where there was a moderate amount of dilation, atrophic changes are noted. *a* is the slit occupied by the band; *b*, the vessel wall proximal to the band; *c*, the vessel wall distal to the band.

PLATE 24.

FIG. 10. Dog 2, Series I. The band was tightened until the pulse had just disappeared. When examined after 7 months, a solid fibrous, cylindrical cord was found under the band.

PLATE 25.

FIG. 11. Dog 2, Series I. Longitudinal section through the fibrous cord shown in Fig. 10. Weigert's elastic tissue stain. *a* is the site of the band, under it the elastic tissue has vanished; *b*, the vessel wall proximal to the band; *c*, the vessel wall distal to the band, showing definite atrophy of the elastic tissue; *d* to *d*,' the fibrous cord; *e*, fibrous tissue that formed about the band. There is almost complete disappearance of the elastic tissue at the site of the band.

PLATE 26.

FIG. 12. Dog 2, Series I. Transverse section of the fibrous cord through *d*, Fig. 11. Hematoxylin and eosin.

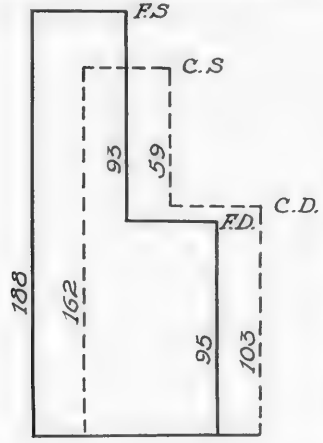

FIG. 1.

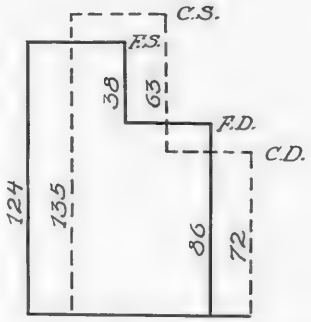

FIG. 2.

F.S. 188. F.D. 95. Pulse pressure 93.
C.S. 162. C.D. 103. Pulse pressure 59.

F.S. 124. F.D. 86. Pulse pressure 38.
C.S. 135. C.D. 72. Pulse pressure 63.

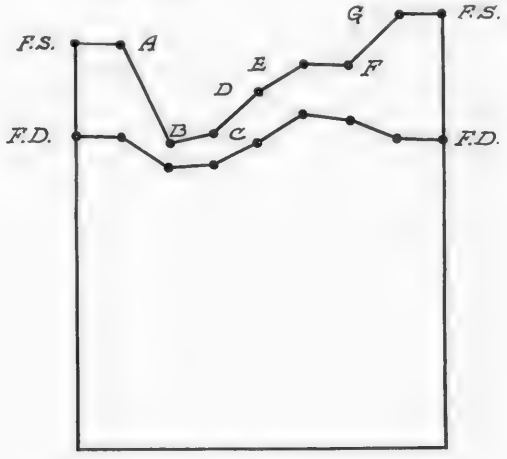

FIG. 3.

A. Without band 175, 135.
B. Immediately after band was applied 131, 121.
C. 5 minutes later 136, 123.
D. 25 minutes later 155, 133.
E. 35 minutes later 167, 144.
F. 35 + minutes later 167, 143.
G. Band removed 189, 138.

(Reid: Partial Occlusion of the Aorta.)

FIG. 4.

FIG. 5.

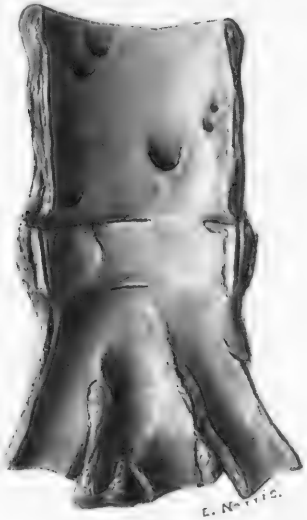

FIG. 6.

(Reid: Partial Occlusion of the Aorta.)

FIG. 7.

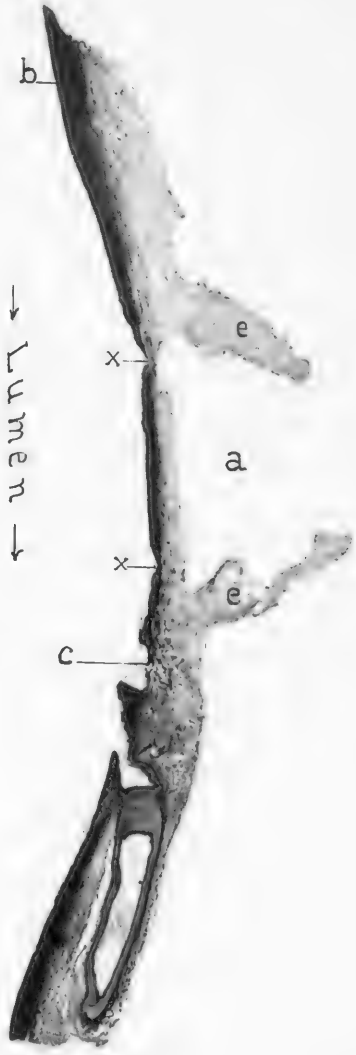

FIG. 8.

(Reid: Partial Occlusion of the Aorta.)

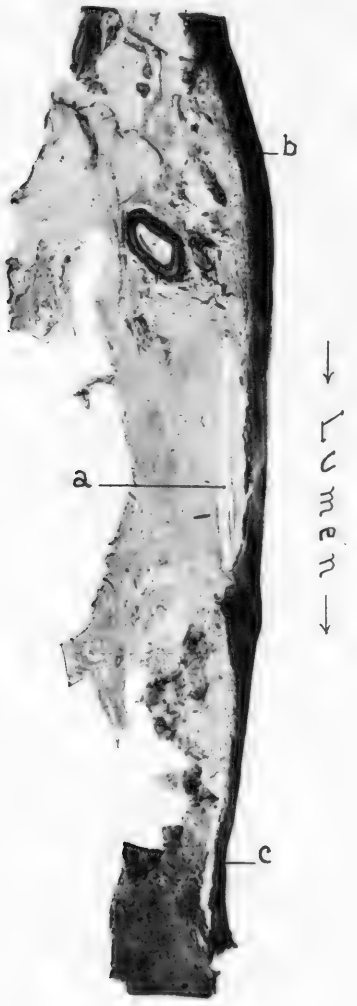

FIG. 9.

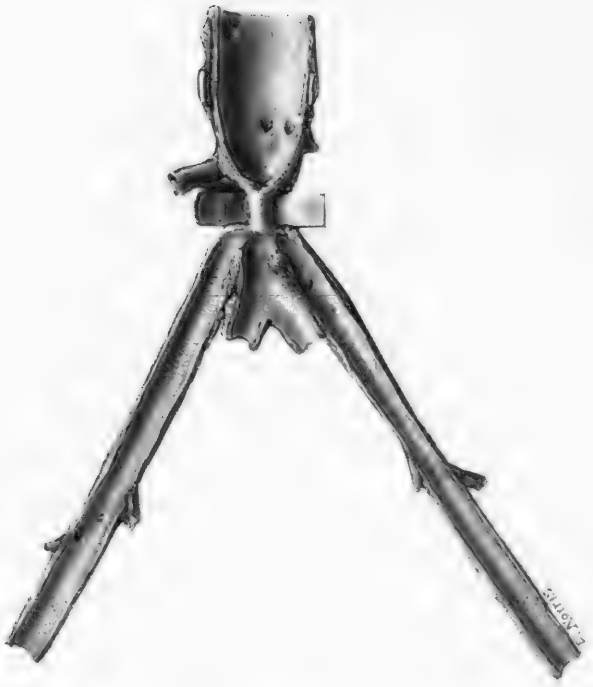

FIG. 10.

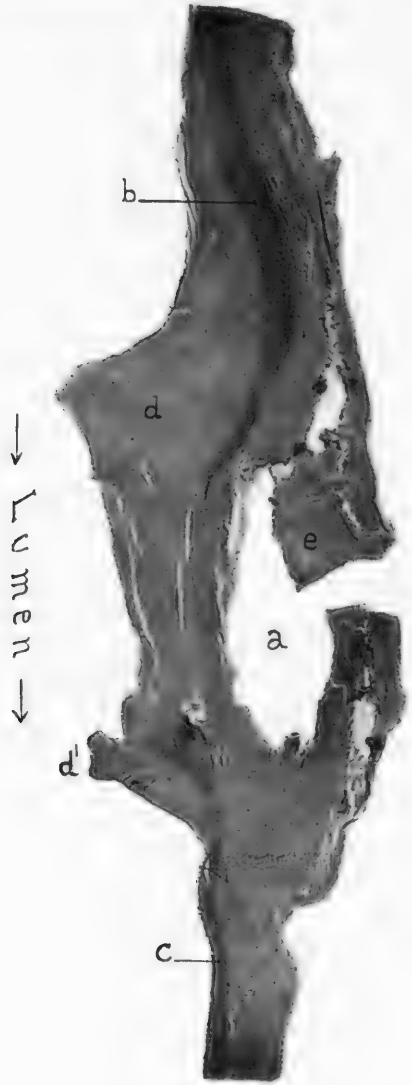

FIG. 11.

(Reid: Partial Occlusion of the Aorta.)

FIG. 12.

(Reid: Partial Occlusion of the Aorta.)

DISTORTION OF THE MALARIAL PARASITE. AN INTERPRETATION OF "PLASMODIUM TENUE" (STEPHENS.)*

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Plates 27 to 35.

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Types of Malarial Parasites.

Practically all authorities agree that there are at least three distinct species of malarial parasites: the tertian parasite, the quartan parasite, and the æstivo-autumnal parasite. This classification is based not only on the morphological and biological characteristics, but also on the clinical manifestations. A few observers have divided the æstivo-autumnal fevers into two groups, one causing quotidian fever, the other malignant tertian. But no convincing morphological basis has been established for making this distinction, and Marchiafava and Bignami,[1] who have so divided the æstivo-autumnal fevers, admit that they are "to be distinguished from each other not so much by their morphological characters as by the mode of their pathogenic action upon man."

Malarial Parasites Recently Described as New Types.—In 1914 papers were published describing certain malarial parasites which were believed by the authors to be new varieties. Emin[2] describes a parasite which he has named *Plasmodium vivax, variete minuta,* but it is not my purpose to discuss Emin's parasite in this paper.

Stephens[3] named the parasite described by him *Plasmodium tenue.* A little

* Aided by a grant from The Rockefeller Institute for Medical Research.

[1] Marchiafava, E., and Bignami, A., Malaria, in Stedman, T. L., Twentieth Century Practice, New York, 1900, xix, 29.

[2] Emin, A., Une variété nouvelle du parasite de Laveran, *Bull. Soc. path. exot.*, 1914, vii, 385.

[3] Stephens, J. W. W., A New Malarial Parasite of Man, *Ann. Trop. Med. and Parasit.*, 1914, viii, 119.

later he[4] published another article apparently referring to parasites similar to those described by him under that name.

In order to make a correct interpretation of the parasites described by Stephens, there are three facts that the observer must bear in mind: (1) that the malarial parasite is attached to corpuscular mounds on the external surface of the red corpuscle; (2) that more than one parasite may be attached to a single corpuscular mound; (3) that the nuclei of young parasites contain a large amount of chromatin.

The Extracellular Relation of the Malarial Parasite to the Red Corpuscle.[5]

All malarial parasites are extracellular throughout their entire life cycle. The following figures show the external relation of the parasite to the red corpuscle. (*a*) Parasites attached to peripheral corpuscular mounds (Figs. 3, 4, 5, 7, 11, 15 to 23, 42, 51, 56, 110, to 112, 168, 237, 238, 242, and 253); (*b*) parasites on the periphery of the infected red corpuscles (Figs. 6, 9, 10, 21 to 33, 36 to 47, 49, 50, 54, 57 to 62, 66, 79, and 239 to 248; (*c*) parasites attached to the upper or under surface of red corpuscles with nuclei or portions of their cytoplasm extending beyond the periphery of the corpuscles to which they are attached (Figs. 1, 2, 48, 52, 53, 55, 63 to 65, 67, 74, 76, 84 to 87, 91, 161 at o, 164, 203 at o, 249, 250, 254, and 264); and (*d*) red corpuscles with Schüffner's granules but no parasites (Figs. 68, 69 at o, 70, and 71).

(*a*) Malarial parasites capture and attach themselves to the outer surface of the red corpuscles by means of pseudopodia arising from the cytoplasm of the parasites. They maintain their position on the external surface of the corpuscles by encircling one or more mounds of hemoglobin substance squeezed up by the parasites for the purpose of attachment. This interpretation of the relation of the para-

[4] Stephens, On the Peculiar Morphological Appearances of a Malarial Parasite, *Ann. Trop. Med. and Parasit.*, 1915, ix, 169.

[5] The author has shown in previous publications that the malarial parasite is extracellular. See Rowley-Lawson, M., *Arch. Int.* Med., 1912, ix, 420; *J. Exp. Med.*, 1913, xvii, 324; 1914, xix, 450; 1915, xxi, 584.

sites to the corpuscles is verified by the mounds being encircled by the parasites at the periphery of the corpuscles. These parasites admit of no other interpretation than that they are on the outer surface of the corpuscles, and it seems unreasonable to suppose that they would use this method of attachment only when situated at the periphery of the corpuscles. Moreover, it must be remembered that this method of attachment gives the only rational explanation of the ring-forms.

(*b*) Parasites resting on the periphery of the red corpuscles with their pseudopodia encircling peripheral or surface mounds.

(*c*) Parasites with nuclei or a portion of their cytoplasm extending beyond the periphery of the corpuscles to which they are attached, show these conditions so clearly that even the most confirmed believer in the intracellular theory must acknowledge that they are on the external surface of the corpuscles. It is the nucleus of the parasite that is most commonly seen projecting beyond the periphery of the infected corpuscle. This occurs when the parasite is attached to a mound at or near the periphery of the corpuscle (Figs. 1 to 3, 7, 18, 19, 50, 53, 67, 84, 91, 203 at o, and 237).

Parasites partly off corpuscles or parasites attached to the surface of corpuscles with their bodies resting on the periphery, are most commonly found at the time of a parasitic migration when the parasite is either abandoning the dehemoglobinized corpuscle or attaching itself to a fresh red corpuscle (with normal color).

Occasionally a parasite may have its nucleus or a portion of its cytoplasm drawn off the infected corpuscle by technique without appreciable injury to the corpuscle (Figs. 46 to 49, 51 to 53, 55, 56, 58, 62, 74, 76, 85 to 87, 161, 164, 246, 249, 253, 254, and 264).

(*d*) Red corpuscles showing Schüffner's granules but having no parasites attached to them may occasionally be found in malarial infections (Figs. 68 to 71).

Schüffner's granules differ in morphology and staining reaction from Maurer's dots and rings, from the blue stippling of red corpuscles, and from the granular corpuscular skeletons. The following figures show that the granulations of these corpuscles without parasites are not to be confounded with other stippling or granula-

tion: (*a*) a red corpuscle with Schüffner's granules and no parasite next to a red corpuscle with Schüffner's granules and a parasite (Fig. 71); (*b*) a red corpuscle with Schüffner's granules and no parasite next to a red corpuscle with blue stippling (Fig. 69); (*c*) a red corpuscle with Schüffner's granules and a parasite next to a corpuscular skeleton or dehemoglobinized red corpuscle (Fig. 73); (*d*) three red corpuscles showing Maurer's dots and rings (Figs. 78 to 80). Two of these corpuscles are infected by æstivo-autumnal parasites (Figs. 78 and 79), and one is infected by two young quartan parasites (Fig. 80).

All observers at present agree that Schüffner's granules are always associated with corpuscles which harbor malarial parasites; and that the granules are the effect of the actual presence of the parasites; that is, the parasites must have been attached to these corpuscles before the granulation could have occurred.

It might be suggested that these granules resulted from something other than the actual presence of an invading parasite; but such an hypothesis is highly improbable. The natural supposition would be that the parasites had been attached to the corpuscles long enough to cause the granulation, and then, for some reason had abandoned them. If this is true, the invading parasites must have been attached to the external surface in order to have escaped without visible injury to the contour of the infected corpuscles.

Ever since Marchiafava and Celli[6] decided that malarial parasites were intracellular because they never observed the pseudopodia beyond the periphery of the infected corpuscles, few observers have ventured to dispute their theory. Mannaberg[7] did not consider the young forms to be intracellular, and in referring to Marchiafava and Celli's findings, he states: "The criticisms brought forward by Marchiafava and Celli are not a confirmation of their view, for the adhesiveness of the parasites would prevent their ever, even during ameboid movement, becoming free from the blood corpuscles."

Schaudinn[8] pictures an adult tertian parasite with a portion of its cytoplasm

[6] Marchiafava and Celli, quoted from Marchiafava and Bignami, Malaria, in Stedman, T. L., Twentieth Century Practice, New York, 1900, xix, 29.

[7] Mannaberg, J., Malarial Parasites, London, 1894, 276.

[8] Schaudinn, F., Studien über krankheitserregende Protozoen. II. *Plasmodium vivax* (Grassi & Feletti), der Erreger des Tertianfiebers beim Menschen, *Arb. k. Gsndhtsamte.*, 1902, xix, 169, and Plate V, Fig. 86.

extending beyond the periphery of the infected corpuscle. Gautier[9] depicts the appearance of the ring projecting beyond the cell. And Ewing[10] states: "It is generally accepted that the tertian parasite lies within the red cell yet, in many tertian cases the body and especially the nucleus of the parasite appear to project beyond the border of the cell, even more distinctly than in the case of the æstivo-autumnal ring." Loeffler[11] shows in a colored plate three young æstivo-autumnal parasites, the nuclei of which project beyond the periphery of the infected corpuscle.

Multiple Infection of Corpuscular Mounds.

The second fact to bear in mind in order to interpret successfully Stephens' *Plasmodium tenue* is that more than one parasite may occupy one corpuscular mound.

When more than one parasite is attached to one corpuscular mound, and the cytoplasm of the last parasite to attach itself is superimposed over that of the other parasite or parasites, one may get the impression of a single ring with several masses of chromatin. This is especially true in the case of the young parasite where practically all the cytoplasm is required to enable it to encircle a mound (Figs. 2, 8, 14, 119 at o, 125 at o, and 143 at o).

This occurrence has been variously interpreted by many observers. Emin,[12] in the description of his new parasite, gives examples of two and three young parasites occupying one corpuscular mound and interprets the condition as that of a precocious division of the chromatin.

Many of the pictures of multiple infection of corpuscular mounds were selected so that the parasites occupying that position could readily be recognized as individuals (Figs. 7, 10 to 13, 22, 34, 35, and 113), and not be interpreted as conjugating parasites or single parasites with more than one chromatin mass.

[9] Gautier, quoted from Ewing, J., Malarial Parasitology, *J. Exp. Med.*, 1900–01, v, 474.

[10] Ewing, Malarial Parasitology, *J. Exp. Med.*, 1900–01, v, 474.

[11] Loeffler, F., Malarial Diseases, in Modern Clinical Medicine, New York, 1910, Colored Plate H.

[12] Emin, Une variété nouvelle du parasite de Laveran, *Bull. Soc. path. exot.*, 1914, vii, Plate IV, Figs. 3 to 5.

The Large Amount of Chromatin in the Nuclei of Young Parasites.

The third fact to remember is the large amount of chromatin which the nuclei of young parasites contain. I think that this fact is readily recognized by most observers. To illustrate this I have selected pictures of young æstivo-autumnal parasites attached to red corpuscles with their nuclei drawn out by technique beyond the periphery of the corpuscles to which the parasites are attached (Figs. 85 to 87, and 264).

These examples serve to illustrate not only the large amount of chromatin in the nuclei, but prove the external relation of these parasites to the red corpuscles; for were they within the substance of the corpuscles, such a condition as that pictured would obviously be impossible.

The entire set of nuclei from a segmenting tertian organism forcibly removed from the parasites with strings of chromatin drawn out by technique (Fig. 83) also serves to illustrate the enormous amount of chromatin contained in the nuclei of young parasites.

Parasites Distorted by Technique (*Plasmodium tenue*). (*Figs. 92 to 269.*)

Several observers have written about Stephens' *Plasmodium tenue* without giving a rational explanation of the peculiar appearances presented by the parasites illustrated, but criticizing him for drawing his conclusions from a single blood film. I shall now give what I believe to be the true interpretation of the forms that he shows.

Stephens drew his conclusions wholly from the peculiar morphological appearances of the parasites which he has demonstrated in both of his articles. I judge that he believes the malarial parasite to be intracellular. He states in his second article: "forms are found in which chromatin alone without any protoplasm occurs in the red cell;" and he also suggests that the parasites cannot be distorted by technique without apparent distortion of the infected red corpuscle. In the same article he writes: "I would point out that the blood cells present no evidence whatsoever of stretching or distortion," and if the parasites he pictures were within the substance of the red corpuscle they could not be distorted by technique and the corpuscle retain its normal contour.

The unusual appearances presented by the parasites he pictures are due entirely to technique. They are young, unpigmented parasites attached to the external surface of the red corpuscles and drawn out of shape during the process of spreading the blood film. I have seen these forms many times.

A careful study of the parasites resting on the periphery of the corpuscles shown in the accompanying plates will suggest the ease with which they could be distorted, and it is surprising that we so often get normal-appearing parasites with our crude methods of spreading films. Possibly the corpuscular mounds, to a certain extent, protect the parasites from distortion; but while it is comparatively easy to distort parasites, they are not easily dislodged.

Distorted parasites are produced accidentally; that is, you may get them even with the most careful technique. Violent technique usually distorts the corpuscles as well as the parasites. With a heavy infection, the blood may not spread freely and may stick slightly when the cover-slips are drawn apart, thus distorting the parasites. Occasionally parasites are distorted if uneven pressure is used in spreading the blood on a slide. They are most commonly found in that part of the specimen where the film is unevenly spread, and along the edges of films made on slides and cover-slips. They may be found where the film is thickly spread as well as where it is thin. If thoroughly examined, almost any specimen of malarial infection containing young parasites, will show a few distorted forms. It is rare to find a specimen in which the greater number of parasites is markedly distorted, but I have seen a few specimens where the normal parasite was the exception. Stephens states in both articles that ring-forms were present in his specimens. "Forms resembling 'rings' do occur," and "it is possible to trace a transition from normal ring-forms," etc.

Types of Distorted Parasites.—In general three types of distorted parasites may be recognized: (*a*) parasites with nuclei distorted and cytoplasm unaltered or but slightly distorted (Figs. 105 at o, 117 at o, 125 at x, 126 to 129, 131 at x, 135, 138, 139, 140 at x, 141, 145 at x, 147, 149, 153, 154 at x, 169, 171, 172, 191, and 192); (*b*) parasites with cytoplasm distorted and nuclei intact or but little disturbed (Figs. 98 at x, 102 at x, 130, 156, 158, 160, 161, 164, 165, 178, 183,

193, 194, 195, 196, 197, 207, 210, 211, 226 at x, and 232 to 236); (*c*) parasites with nuclei and cytoplasm markedly or slightly distorted (Figs. 131 to 133, 136, 137, 140, 142, 144 to 146, 148, 150 to 152, 154, 155, 157, 159, 162, 166 to 168, 170, 173 to 177, 179 to 181, 184 to 190, 199 to 204, 206, 208, and 212 to 231).

Evidence That the Appearance Presented by the Parasites Described by Stephens Is Due to Technique.—If one examines a specimen showing a rich infection and containing many of the forms which I have described as distorted parasites, one will find certain parts of the film where the parasites are drawn out, in one general direction, while the infected corpuscles, although they may be flattened out, retain their normal contour (Figs. 102, 148, 150 to 152, and 156 to 160). And groups of parasites will be found with their nuclei similarly distorted (Figs. 147, 149, 153, and 167).

These facts should suggest technique as the cause, provided that one is willing to concede that the parasites are attached to the external surface of the red corpuscles. No other explanation seems possible, since bizarre parasites could not be limited to certain parts of the blood film.

When two or more parasites attached to one corpuscle are seen stretched across the corpuscle, in one direction (Figs. 102, 150, 152, 155, 156, 158, 165, 166, 171, 172, 177 to 180, 188, 189, 197, 222, and 227 to 230), technique should suggest itself as the cause.[13]

Distortion of Parasites Occupying One Corpuscular Mound.—Instances of two or more parasites occupying one corpuscular mound, when the parasites are distorted by technique, present appearances such as may be seen in Figs. 117 at o, 128 at x, 129 to 131, 138, 139, 142, 165, 166, 169, 170, 180, 195, 198, 206, 207, 209 to 212, 222, and 226 at x.[14]

Fusion of Chromatin.—The nuclei of two or more parasites may be so fused together by technique as to give the appearance of a single mass or string of chromatin. This may be seen in Figs. 105

[13] Stephens shows this condition in Figs. 19, 26, 27, 30, 31, and 32, of the colored plate illustrating his first article.

[14] This is also shown in Figs. 2 to 18, 20, 23 to 25, 28 to 31, and 33 to 35 of the colored plate illustrating Stephens' first article.

at o, 117 at o, 127 at o, 129, 139, 142, 166, 169 at o, 192, 195, 198, 206, 209, 210, and 212.[15]

How to Obtain Distorted Parasites So That They May Be Identified as Such.—Take several films from one large drop of blood, using varying technique in preparing them. It is more satisfactory to work this out with a heavy infection, showing several parasites in one field. One may have to make films from many specimens to obtain distorted parasites, or one may secure them in the first attempt. Distorted parasites in one or two of a set of films and none in the others indicate that the distortion is due to technique.

I have several times secured distorted parasites in this manner, accidentally. I frequently take four cover-slips from one drop of blood in order to study various phases in the life of the parasite. Occasionally one of the specimens contains certain distorted parasites, while in the other specimens the parasites are normal. Without other evidence, this would be proof that these forms are due to technique.

Plasmodium tenue.—This is an appropriate description of the drawn out, flattened parasite. As one would expect, it shows but little density compared with the normal parasite; the bulk of cytoplasm varies with the age of the parasite. Stephens writes: "The parasite has but little bulk, or density."

Many of the distorted æstivo-autumnal parasites in Figs. 115, 117 and 118 to 146 show but little cytoplasm compared with the amount of chromatin. These parasites are very young. (The normal parasites from the same film may be seen attached to corpuscles in Figs. 1 to 8, and free in Figs. 118 to 124.) The specimen containing these parasites was taken 15 minutes after a paroxysm, and contained, besides the parasites attached to the red corpuscles, segmenting bodies, free segments, phagocytes with inclusions of segmenting bodies and pigment masses, as well as free pigment masses. Two cover-slips were taken from one drop of blood; one contained the distorted forms shown, the other normal parasites.

The distorted æstivo-autumnal parasites shown in Figs. 168 to

[15] This may also be seen in Figs. 2 to 4, 7, 12, and 16 of the colored plate accompanying Stephens' first article.

181 were taken from a specimen of blood secured 1 hour after a paroxysm. Figs. 9 to 14 show normal parasites attached to corpuscles, which were taken from the same specimen. The distorted æstivo-autumnal parasites shown in Figs. 147 to 167 were from the same case as the normal parasites in Figs. 15 to 34. The distorted quartan parasites in Figs. 183 to 192 correspond in age to the quartan parasites in Fig. 80.

Adult as well as young parasites may be flattened and drawn across the corpuscle by technique. The tertian forms (Figs. 232 to 236) were from one film and were distorted in the same general direction. This type of distorted parasite corresponds to the so called quartan "*bandform*" parasite pictured by Ruge,[16] Kolle and von Wassermann,[17] Ziemann,[18] Loeffler,[19] von Wasielewski,[20] and others.

Red Corpuscles to Which Distorted Parasites Are Attached.—Parasites may be more or less distorted without apparent flattening of the infected corpuscle, or change in its circular contour; but this is not the rule. Red corpuscles to which these parasites are attached, usually appear to be ironed out, and the adjacent uninfected corpuscles may give the same appearance. Practically all the infected red corpuscles shown in both of Stephens' articles have a slightly irregular outline and appear to be flattened out. This makes it difficult to ascertain whether the distorted parasites enlarged the infected corpuscles or otherwise, and it explains why Stephens found it difficult to determine whether the *Plasmodium tenue* enlarged the corpuscle or not. He writes: "I am not sure whether this parasite enlarges the cell, as, although in some cases I found infected cells larger than non-infected ones in their vicinity, in other cases the reverse held good."

[16] Ruge, R., Einführung in das Studium der Malariakrankheiten mit besonderer Brücksichtigung der Technik, Jena, 1901, Plate I, Fig. 16.

[17] Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Atlas, Jena, 1902, Plate III, Fig. 65.

[18] Ziemann, H., Malaria, in Mense's Handbuch der Tropenkrankheiten, Leipzig, 1906, iii, 269; Plate IX, Figs. 39 and 41.

[19] Loeffler, Malarial Diseases, in Modern Clinical Medicine, New York, 1910, Plate I (C), Figs. 2 and 3.

[20] von Wasielewski, T., Plasmodiden, in Rubner, Gruber, and Ficker's Handbuch der Hygiene, 3te Abt., Leipzig, 1913, iii, Plate 26, Fig. A, 4, 6.

Stephens' Forms in Which Chromatin Particles or Strands without Protoplasm Occur in the Red Cell.—Stephens refers to this condition in his second article. His views as to the probable nature of the forms he describes are as follows:

"(1) That they are a new species of parasite.

"(2) That they are degenerative, i.e., formed in the body under unknown conditions, and so perhaps analogous to the so-called quinine forms of parasites.

"(3) That they are artificial, i.e., formed outside the body under unknown conditions."

And he goes on to say: "The fact that among the parasites figured, forms are found in which chromatin alone without any protoplasm occurs in the red cell is, I think, in favor of, but not decisive for, one of the latter two views rather than the first." He states that forms consisting of protoplasm alone, without chromatin, were not seen.

The chromatin masses without any cytoplasm which he describes as being present, are, I believe, stained remnants of parasites whose cytoplasm has faded.

Parasites Whose Chromatin or Cytoplasm Has Faded or Failed to Stain.—There are two general types of partially stained parasites which may be described as follows: (*a*) parasites whose chromatin remains stained, but whose cytoplasm has faded or failed to stain (Figs. 88 to 90, 101, 105 to 109, 111, 112, 114, 116, 266, 267, and 269); and (*b*) parasites whose cytoplasm remains stained, but whose chromatin has faded or failed to stain (Figs. 92 to 97, 100 at o, 102 at o, 103, 104, and 268).

The examples of *a* and *b* shown herewith were taken from specimens containing successfully stained parasites. It is rare to find examples of both *a* and *b* in one film unless it is fading rapidly.

One may find parasites without nuclei on corpuscles to which are attached parasites with well stained nuclei (Figs. 92 to 97, and 100). That these nuclei might have been removed from the parasites by technique was considered; but as the gradual fading of the chromatin of various parasites could be followed, and a careful search failed to reveal free nuclei, the only reasonable conclusion to be reached was that the chromatin had faded. The gradual fading of cytoplasm may be followed in the same way.

Stained nuclei of parasites whose cytoplasm has faded are shown well in Figs. 88 to 90. Here the nuclei are successfully stained, as well as the crescents seen near them. Well stained young parasites were seen in the same film (Fig. 91).

Distorted chromatin of partially faded parasites may be seen in Figs. 105 to 109, 112, and 116, and chromatin which has not been distorted may be seen in Figs. 111 and 114.

If specimens showing the above conditions are restained, it is often possible to bring out the cytoplasm in connection with the chromatin, or the chromatin in connection with the cytoplasm.

Species of Parasite Described in Stephens' First Article.—The distorted parasites described by Stephens as *Plasmodium tenue* might be tertian, quartan, or æstivo-autumnal parasites; but judging from the delicate morphology of the parasites and the numerous instances of multiple infection of corpuscular mounds and of red corpuscles, I should judge them to be of the æstivo-autumnal type. In my experience the multiple infection of red corpuscles by young parasites is much more common in the æstivo-autumnal than in other malarial infections.

In the last paragraph of Stephens' second article, he refers to "further films from the case of *P. tenue*. Two films (March, 1914) showed quartan, and in one of the films a single pigmented (presumably simple tertian) parasite was found with the pigment in the form of rods, in an enlarged cell showing Schüffner's dots. Three films (June) showed quartan, and one (July), quartan." I should not say that this was evidence that the first specimen containing *Plasmodium tenue* forms was from a case of quartan infection.

The specimen containing the *Plasmodium tenue* forms described by Stephens in his first paper, was sent to him in the autumn of 1913, and if this case remained untreated or infected until March, 1914 (the first date stated above), one could not, after this lapse of time, be sure of dealing with the same infection unless one could eliminate the possibility of a reinfection by other species of malarial parasite. This would be especially true were the patient living in a malarial district.

The type of infecting parasite cannot always be designated by the occasional presence of Schüffner's granules or Maurer's rings in

the infected red corpuscle. The following figures are evidence of this: (*a*) a crescent attached to a red corpuscle showing Schüffner's granules (Fig. 77); (*b*) a quartan parasite attached to a red corpuscle showing Schüffner's granules (Fig. 81); (*c*) two young quartan parasites attached to a red corpuscle showing Maurer's rings and dots (Fig. 80). These are shown in connection with a crescent and an æstivo-autumnal ring-form parasite attached to red corpuscles showing Maurer's rings (Figs. 78 and 79). And one occasionally sees æstivo-autumnal ring-forms attached to red corpuscles showing Schüffner's granules.

Pigment of Malarial Parasites.—It should be mentioned here that too much importance must not be attached to the form which the pigment granules present in the parasites, for this pigment is subject to distortion, just as the parasites are, and it may be distorted without distortion of the parasites, just as the parasites may be distorted without distortion of the infected corpuscles. Much of the rod-shaped pigment seen owes its rod shape to technique.

Distorted Parasites Previously Described.

Previous to Stephens' two articles describing distorted parasites, several papers came out illustrating various types of parasites distorted by technique. In 1904 the author[21] published an article entitled, "Unusual Forms of Malarial Parasites," in which were pictured elongated forms of malarial parasites from æstivo-autumnal infections, wrongly interpreting these forms as stages in the life cycle of the crescent. In 1906 Ziemann[22] called attention to distorted forms of the æstivo-autumnal parasite. In 1908 Balfour and Wenyon[23] pictured distorted forms of the æstivo-autumnal parasites, interpreting them as ameboid forms. In 1911 in an article on the æstivo-autumnal parasite, the author[24] pictured various types of distorted parasites, stating that they were the result of technique.

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[21] Rowley, M. E., Some Unusual Forms of Malarial Parasites, *Bull. Johns Hopkins Hosp.*, 1904, xv, 22.

[22] Ziemann, Malaria, in Mense's Handbuch der Tropenkrankheiten, Leipzig, 1906, iii, 294.

[23] Balfour, A., and Wenyon, C. M., Sanitary Notes. Khartoum, *Third Rep. Wellcome Research Lab., Gordon Memorial College, Khartoum*, 1908, Colored Plate 7.

[24] Rowley-Lawson, M., The Æstivo-Autumnal Parasite: Its Sexual Cycle in the Circulating Blood of Man, with a Description of the Morphological and Biological Characteristics of the Parasite, *J. Exp. Med.*, 1911, xiii, 263.

In 1913 Sergent, Beguet, and Plantier[25] published an article in which attention is called to young æstivo-autumnal parasites with streaked arrangement of the chromatin. These forms are due to technique.

In 1914 Stephens published his article on distorted forms, "*P. tenue*," and this called out criticism and other illustrations of distorted parasites from Balfour and Wenyon[26] and Ziemann,[27] although these authors did not interpret the forms as due to technique. But in their last paper Balfour and Wenyon recognize that "it may be possible to find for these variations some mechanical explanation." In 1901 Ewing[28] mentions, in connection with the æstivo-autumnal ring-form parasite, that "occasionally such rings were found to have unfolded and to be stretched like a thread across the cell, the nuclei appearing at inconstant intervals." And in connection with the tertian ring-form parasite, he[29] writes: "Elongated forms of the young parasite are often seen in which the ring is absent and the nuclear body lies bare at one end." He undoubtedly refers here to parasites distorted by technique.

SUMMARY.

1. The correct interpretation of the form described by Stephens under the name of *Plasmodium tenue* is as follows: It is not a new species of parasite or an ameboid form, but a parasite attached to the external surface of red corpuscles and distorted by technique.

2. Evidence against a new species of parasite is: (*a*) they may be found in all known malarial infections and at any stage in the development of the parasite; and (*b*) they show evidence of distortion.

3. Evidence against ameboid forms and in favor of parasites distorted by technique is: (*a*) they may be found in but one of the two cover-slips prepared simultaneously; (*b*) they may be found in certain definite groups in various parts of a film which otherwise contains normal appearing parasites; (*c*) in rich infections, containing many disforted forms, they may be found in certain parts of a film, all distorted in one direction, the infected corpuscles and those adjacent to them showing no evidence of injury; and (*d*) two or more

[25] Sergent, Edw., Sergent, Et., Beguet, M., and Plantier, A., Observations microscopiques au cours d'un acces pernicieux paludées, *Bull. Soc. path. exot.*, 1913, vi, 615.

[26] Balfour and Wenyon, The So-Called *Plasmodium tenue* (Stephens), *J. Trop. Med.*, 1914, xvii, 353.

[27] Ziemann, Ueber eigenartige Malariaparasitenformen, *Centr. Bakteriol., 1te Abt., Orig.*, 1915, lxxvi, 384.

[28] Ewing, Malarial Parasitology, *J. Exp. Med.*, 1900–01, v, 446.

[29] Ewing, Malarial Parasitology, *J. Exp. Med.*, 1900–01, v, 436.

parasites attached to one red corpuscle may be seen to be distorted in one direction, or to have nuclei similarly distorted.

4. Three general types of distorted parasites may be recognized: (*a*) parasites with nuclei distorted and cytoplasm unaltered or but slightly distorted; (*b*) parasites with cytoplasm distorted and nuclei intact or but little disturbed; and (*c*) parasites with both nuclei and cytoplasm slightly or markedly distorted.

5. Red corpuscles to which distorted parasites are attached usually appear to be flattened out with slightly irregular outline, but parasites may be more or less distorted without any apparent change in the infected corpuscle.

6. Parasites with well stained chromatin and faded cytoplasm, or parasites with well stained cytoplasm and faded chromatin, may be found in specimens containing well stained parasites. It is usually possible to restain these specimens bringing out the faded cytoplasm belonging to the well stained chromatin or the lost chromatin belonging to the well stained cytoplasm.

EXPLANATION OF PLATES.

PLATE 27.

ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,680. Tertian parasite (Fig. 35).

Figs. 1 to 8. Parasites secured 15 minutes after a paroxysm.

Figs. 9 to 14. Parasites secured 1 hour after a paroxysm.

Figs. 15 to 35. Parasites secured 4 hours after a paroxysm.

FIG. 1. Parasite attached to the surface of a corpuscle with a nucleus extending beyond the periphery of the corpuscle.

FIG. 2. Two parasites attached to one corpuscular mound, the nucleus of one parasite extending beyond the periphery of the corpuscle. It requires the entire cytoplasm of each parasite to encircle the mound, so that the cytoplasm of one parasite being superimposed over that of the other parasite gives the appearance of a single parasite with two masses of chromatin.

FIGS. 3 to 5. Parasites attached to peripheral corpuscular mounds; the nuclei of these parasites extend beyond the periphery of the corpuscles to which they are attached.

FIG. 6. Parasites resting on the periphery of the corpuscles to which they are attached. Cytoplasm as well as nuclei may be seen extending beyond the periphery of the corpuscle.

FIG. 7. Two parasites attached to a peripheral corpuscular mound and two parasites attached to a surface mound. The nuclei of the parasites attached to the peripheral mound may be seen extending beyond the periphery of the corpuscle.

FIG. 8. Parasites attached to surface mounds. At the left two parasites encircle one mound; their cytoplasm being superimposed, they give the appearance of a single parasite with two masses of chromatin.

FIG. 9. Parasites attached to decolorized peripheral mounds. Portions of these parasites extend beyond the periphery of the corpuscle.

FIG. 10. Two parasites attached to one corpuscular mound; the cytoplasm of the upper parasite extends beyond the periphery of the corpuscle.

FIG. 11. Two parasites attached to a peripheral corpuscular mound, and two parasites of a slightly larger growth, attached in a similar manner to a surface corpuscular mound.

FIGS. 12 and 13. Two parasites attached to one corpuscular mound. These parasites may be seen to be individuals.

FIG. 14. Four parasites attached to one corpuscular mound. A careful scrutiny will enable one to trace each parasite.

FIGS. 15 to 20. Parasites attached to peripheral corpuscular mounds. The nuclei of the parasites in Figs. 18 and 19 may be seen extending beyond the periphery of the corpuscles.

FIG. 21. The parasite rests on the periphery of the corpuscle, and the pseudopodium may be seen encircling the peripheral corpuscular mound.

FIG. 22. Two parasites encircling one peripheral corpuscular mound.

FIG. 23. The parasite rests on the periphery of the corpuscle and encircles a corpuscular mound in front of it.

FIGS. 24 to 33. Parasites resting on the periphery of the corpuscles with pseudopodia encircling the surface corpuscular mounds.

FIG. 34. Two parasites encircling one decolorized corpuscular surface mound.

FIG. 35. Three tertian parasites attached to a red corpuscle. One parasite surrounds with its pseudopodium the other parasite.

PLATE 28.

TERTIAN PARASITES, EXTRACELLULAR EVIDENCE.

Magnification, × 1,680.

FIGS. 36 to 41. Young parasites resting on the periphery of the corpuscles to which they are attached.

FIG. 42. A parasite attached to a peripheral corpuscular mound.

FIG. 43. A parasite resting on the periphery of a corpuscle with pseudopodia overlying the corpuscle.

FIG. 44. A parasite attached to a corpuscle. The nucleus and part of the cytoplasm of this parasite may be seen extending beyond the periphery of the corpuscle.

FIG. 45. A parasite resting on the periphery of the corpuscle to which it is attached.

FIGS. 46 to 49. Parasites partly pulled off the corpuscles to which they are attached. Technique is responsible for the dislocation.

FIG. 50. A young parasite attached to a corpuscle. The nuclei of the parasite are seen extending beyond the periphery of the corpuscle.

FIGS. 51 and 52. Two parasites partly removed from the corpuscles to which they are attached. The parasite in Fig. 51 is attached to a peripheral corpuscular mound, the parasite in Fig. 52 to a surface mound.

FIG. 53. A parasite attached to a red corpuscle with nuclei and pseudopodia (at o) extending beyond the periphery of the corpuscle.

FIG. 54. A pigmented parasite which has probably just attached itself to a fresh red corpuscle, the hemoglobin of which appears to be intact.

FIG. 55. A young parasite, unfolded and partly removed from the infected corpuscle by technique.

FIG. 56. A young parasite attached to a peripheral corpuscular mound with its pseudopodium extending beyond the periphery of the corpuscle.

FIG. 57. A pigmented parasite which is probably just attaching itself to a fresh red corpuscle, the hemoglobin of which appears to be intact.

FIG. 58. A young parasite with two pigment granules partly removed from a red corpuscle by technique. I believe that this parasite has recently attached itself as the hemoglobin of the corpuscle appears to be intact, while the parasite is pigmented.

FIGS. 59 to 61. Parasites resting on the periphery of the corpuscles to which they are attached. The parasites in Figs. 59 and 61 are pigmented and older than that in Fig. 60. The latter is unpigmented and flattened out by technique.

FIG. 62. A pigmented parasite partly pulled off the infected corpuscle by technique. This corpuscle shows no loss of hemoglobin though the parasite is heavily pigmented.

FIGS. 63 to 65. Adult pigmented parasites with portions of their cytoplasm extending beyond the periphery of the corpuscles to which they are attached.

FIG. 66. A parasite resting on the periphery of the corpuscle with its pseudopodium encircling a surface mound.

FIG. 67. An adult parasite attached to a corpuscle with the nucleus extending beyond the periphery of the corpuscle.

PLATE 29.

TERTIAN, ÆSTIVO-AUTUMNAL, AND QUARTAN PARASITES.

Magnification, × 1,680.

FIG. 68. A red corpuscle with Schüffner's granules and no parasite, from a case of tertian infection.

FIG. 69. A red corpuscle with Schüffner's granules and no parasite at o next to a red corpuscle with blue stippling, from a tertian infection.

FIG. 70. A red corpuscle with Schüffner's granules and no parasite, from a tertian infection.

FIG. 71. A red corpuscle with Schüffner's granules and no parasite next to a red corpuscle with Schüffner's granules and a tertian parasite.

FIG. 72. A free, distorted ring-form parasite from a tertian infection. The nucleus may be seen at x and a grain of pigment at o.

FIG. 73. A red corpuscle with Schüffner's granules and a tertian parasite next to a granular corpuscular skeleton or dehemoglobinized remnant of a red corpuscle. These examples are shown side by side so that the differences in granulations and staining reaction may readily be noted.

FIG. 74. A tertian parasite partly pulled off a red corpuscle with Schüffner's granules.

FIG. 75. A free, distorted ring-form parasite from a tertian infection. The nucleus may be seen at x and a grain of pigment at o.

FIG. 76. An adult tertian parasite partly pulled off a red corpuscle with Schüffner's granules.

FIG. 77. A crescent attached to a red corpuscle showing Schüffner's granules.

FIG. 78. A crescent attached to a red corpuscle showing Maurer's rings. The infected corpuscle is not entirely decolorized.

FIG. 79. An æstivo-autumnal ring-form parasite attached to a red corpuscle showing Maurer's rings and dots. The magnification of this figure is 2,120.

FIG. 80. Two young quartan parasites attached to a red corpuscle showing Maurer's rings and dots. The parasites are slightly distorted (in one direction) by technique.

FIG. 81. A quartan microgametocyte attached to a red corpuscle showing Schüffner's granules.

FIG. 82. A tertian segmenting body attached to an apparently healthy corpuscle. The chromatin is much distorted by technique without distortion of the infected corpuscle. The light dots are pigment granules that have been partially dissolved by xylol. This parasite must have received its pigment from another red corpuscle or corpuscles.

FIG. 83. A set of nuclei and pigment granules removed from a tertian parasite by technique. This example serves to illustrate the enormous amount of chromatin contained in the nuclei of young parasites. The long strands illustrated are drawn out from the chromatin of the nuclei.

FIG. 84. Two pigmented tertian parasites attached to a fairly healthy-appearing corpuscle. The nucleus of the parasite at the left may be seen to be distorted at x. The nucleus of the parasite at the right may be seen projecting beyond the periphery of the corpuscle at o.

FIGS. 85 to 87. Young æstivo-autumnal parasites attached to red corpuscles with the chromatin of their nuclei drawn out in long thread-like processes beyond the periphery of the corpuscles to which the parasites are attached. These figures serve to illustrate not only the large amount of chromatin in the nuclei

of the young parasites but prove that these parasites are attached to the external surface of the corpuscles. In Fig. 86 at o two tiny parasites surround the corpuscular mound.

FIGS. 88 to 90. Chromatin dots (nuclei) of parasites whose cytoplasm has faded. Well stained crescents may be seen near the nuclei. A twin specimen, which had also faded, was successfully restained.

FIG. 91. A young æstivo-autumnal parasite, well stained, from the same specimen as that containing Figs. 88, 89, and 90.

PLATE 30.

ÆSTIVO-AUTUMNAL PARASITES PARASITES PARTIALLY FADED AND PARASITES DISTORTED BY TECHNIQUE.

Magnification, × 1,680.

FIGS. 92 to 97. Parasites with faded nuclei on corpuscles to which are attached parasites with well stained nuclei. The parasites with faded nuclei may be seen at o. In Fig. 96 at x a free parasite with stained nucleus may be seen.

FIG. 98. A distorted parasite at x on a corpuscle next to a corpuscle to which is attached another distorted parasite whose nucleus has faded until only a small particle of chromatin is to be seen at o.

FIG. 99. A well stained distorted parasite attached to a corpuscle next to a corpuscle to which is attached a parasite whose cytoplasm is fading.

FIG. 100. A parasite with faded nucleus at o attached to a corpuscle on which is a parasite with well stained nucleus and cytoplasm.

FIG. 101. Two parasites with distorted chromatin and fading cytoplasm.

FIG. 102. Two distorted parasites, the nucleus of one being faded, at o, and that of the other fading, attached to a corpuscle, next to a corpuscle to which are attached two well stained distorted parasites at x. Note that these four parasites are all distorted in one direction.

FIG. 103. Two parasites with well stained cytoplasm and faded chromatin.

FIG. 104. A distorted parasite with well stained cytoplasm and faded nucleus.

FIGS. 105 to 109. Chromatin masses of parasites whose cytoplasm has faded. These chromatin masses all show distortion by technique. In Fig. 105 at o may be seen two young parasites attached to one corpuscular mound. The nuclei of these parasites are fused so as to give the appearance of two rings with one mass of chromatin.

FIG. 110. Two young, unfaded parasites attached to a peripheral mound.

FIG. 111. Two parasites with faded cytoplasm attached to a peripheral mound. Compare with Fig. 110.

FIG. 112. A young parasite with faded cytoplasm. The nucleus is distorted and shaped to the peripheral mound to which the parasite is attached.

FIG. 113. Multiple infection of two surface mounds by young parasites.

FIG. 114. Chromatin dots (nuclei) of young parasites whose cytoplasm has faded. These nuclei are normal in shape, and from their position, in relation to each other (Fig. 113), multiple infection of corpuscular mounds is suggested.

FIG. 115. Young parasites distorted by technique.

FIG. 116. Distorted masses of chromatin. The cytoplasm of these parasites has faded.

FIG. 117. Parasites distorted by technique. At o two parasites are attached to one corpuscular mound, the nuclei being fused so as to give the appearance of two rings with a single mass of chromatin.

PLATE 31.

ÆSTIVO-AUTUMNAL PARASITES. FREE PARASITES AND PARASITES DISTORTED BY TECHNIQUE.

Magnification, × 1,680.

These parasites were secured 15 minutes after a paroxysm.

FIGS. 118 to 122. Tiny free parasites. At o in Fig. 119 two parasites may be seen surrounding one corpuscular mound. These parasites are from the same film as the normal and distorted parasites in Figs. 1 to 8.

FIGS. 123 and 124. Tiny free parasites at o, and parasites distorted and attached to corpuscles. In Fig. 124 one of the free parasites may be seen to have a pseudopodium.

FIG. 125. Distorted parasites and a multiple infection of a corpuscular mound at o.

FIGS. 126 to 129. Distorted parasites. The nuclei show more distortion than the cytoplasm. In Fig. 127 fusion of the chromatin of two parasites may be seen at o. In Fig. 128 at x, two parasites with distorted nuclei may be seen occupying one corpuscular mound.

FIG. 130. Multiple infection of corpuscular mounds. The parasites are distorted in one general direction.

FIGS. 131 to 142. Various types of distorted parasites. Figs. 131 and 140 at x show two ring-form parasites occupying one corpuscular mound. The nuclei of these parasites are distorted.

FIG. 143. Multiple infection of a red corpuscle. At o four young parasites may be seen attached to one corpuscular mound.

FIGS. 144 and 145. Distorted parasites.

FIG. 146. A free distorted parasite.

PLATE 32.

ÆSTIVO-AUTUMNAL PARASITES. PARASITES DISTORTED BY TECHNIQUE.

Magnification, × 1,680.

These parasites were secured 4 hours after a paroxysm, and from the same case as the normal parasites seen in Figs. 15 to 34.

FIG. 147. Three young parasites attached to one corpuscle and similarly distorted.

FIG. 148. Distorted parasites. At o a peripheral corpuscular mound may be seen. These parasites are distorted in one general direction.

FIG. 149. Two young parasites attached to one corpuscle and similarly distorted.

FIGS. 150 to 152. Distorted parasites. Each figure shows the parasites distorted in one general direction. At x in Fig. 151 the chromatin is intact and the cytoplasm distorted.

FIGS. 153 and 154. Parasites similarly distorted. At x in Fig. 154 the cytoplasm is normal in appearance, the chromatin distorted.

FIG. 155. Distorted parasites. The extraneous thread at o extends across the field in the same general direction as the distorted parasites.

FIG. 156. Three parasites attached to one corpuscle. All three parasites are distorted in one general direction.

FIG. 157. Three parasites distorted in one general direction.

FIG. 158. Two parasites on one corpuscle. These parasites are distorted in the same general direction as the single parasite attached to the corpuscle at the left.

FIGS. 159 to 162. Parasites distorted in one general direction. In Fig. 161 two parasites are attached to one of the corpuscles. The pseudopodia of one of these parasites may be seen extending beyond the periphery of the infected corpuscle at o.

FIG. 163. A free parasite, normal in appearance, of the same age and from the same film as the distorted parasites.

FIG. 164. A parasite with distorted cytoplasm. A portion of the cytoplasm may be seen extending beyond the periphery of the infected corpuscle at o.

FIG. 165. Parasites with distorted cytoplasm. The parasites are distorted in one direction.

FIG. 166. Parasites with distorted chromatin and cytoplasm. These parasites are distorted in one direction.

FIG. 167. Three parasites similarly distorted.

PLATE 33.

ÆSTIVO-AUTUMNAL, QUARTAN, AND TERTIAN PARASITES DISTORTED BY TECHNIQUE.

Magnification, × 1,680.

Figs. 168 to 182. Æstivo-autumnal parasites secured 1 hour after a paroxysm.

Figs. 183 to 192. Quartan parasites in the same stage of development as the parasites in Fig. 80.

Figs. 193 to 202. Tertian parasites.

FIG. 168. The chromatin of this parasite is distorted to conform to the contour of the decolorized peripheral corpuscular mound.

FIG. 169. At o two young parasites are attached to one corpuscular mound, and the chromatin is distorted and fused so as to give the appearance of two rings with a single mass of chromatin.

FIG. 170. Multiple infection of a corpuscular mound. The cytoplasm of these parasites is more distorted than the chromatin.

FIGS. 171 and 172. Two parasites attached to each corpuscle. The parasites are distorted in one direction.

FIGS. 173 to 176. Parasites with cytoplasm and chromatin distorted.

FIGS. 177 to 180. Two and three (Fig. 180) parasites attached to one corpuscle and distorted in one direction.

FIG. 181. A distorted free parasite.

FIG. 182. A free parasite fairly normal in appearance.

FIGS. 183 to 187. Distorted parasites. The infected red corpuscles appear normal in contour.

FIG. 188. Two parasites attached to one corpuscular mound and distorted, both in one direction.

FIG. 189. Two parasites which were probably attached to one corpuscular mound. These parasites are distorted in one direction.

FIGS. 190 to 192. Distorted parasites.

FIGS. 193 to 196. Parasites with distorted cytoplasm.

FIG. 197. Two parasites attached to one corpuscle. The cytoplasm of these parasites is distorted in one direction.

FIG. 198. Two parasites attached to one corpuscular mound. The cytoplasm is distorted and the nuclei are fused.

FIGS. 199 to 202. Parasites with distorted chromatin and cytoplasm.

PLATE 34.

TERTIAN PARASITES. PARASITES DISTORTED BY TECHNIQUE.

Magnification, × 1,680.

FIG. 203. A distorted and a normal parasite attached to one corpuscle. The nucleus of the normal parasite may be seen to project beyond the periphery of the infected corpuscle at o. A careful scrutiny of the picture will show that this parasite is on the under surface of the infected corpuscle.

FIG. 204. A distorted parasite. A peripheral corpuscular mound may be seen at o. The distorted parasite was probably pulled away from this mound by technique, as the mound is seen to be partly decolorized.

FIG. 205. A normal ring-form parasite from the same specimen as that containing many of the distorted forms.

FIG. 206. Multiple infection of a corpuscular mound. The cytoplasm and chromatin are distorted.

FIG. 207. Multiple infection of a corpuscular mound by two parasites. The cytoplasm is distorted.

FIGS. 208 to 223. Various types of distorted parasites.

FIGS. 224 and 225. Two types of distorted free parasites.

FIG. 226. Parasites with both chromatin and cytoplasm distorted. At x two distorted parasites may be seen occupying one corpuscular mound.

FIGS. 227 to 230. Two parasites attached to each corpuscle. These parasites are distorted in one direction.

FIG. 231. Three or more parasites attached to one corpuscle. These parasites are attached and distorted in such a manner that it is impossible to be sure how many parasites are present.

FIGS. 232 to 236. Adult parasites distorted. These parasites are flattened out and drawn across the infected corpuscle. They correspond to the so called "*bandform*" parasites described by many observers as present in quartan infections.

PLATE 35.

TERTIAN AND ÆSTIVO-AUTUMNAL PARASITES.

Magnification, × 1,680.

FIG. 237. (Corresponds to Fig. 18.) An æstivo-autumnal parasite attached to a peripheral corpuscular mound. The nucleus extends beyond the periphery of the infected corpuscle.

FIG. 238. (Corresponds to Fig. 11.) Æstivo-autumnal parasites. Two are attached to a peripheral corpuscular mound, and two to a surface corpuscular mound.

FIG. 239. (Corresponds to Fig. 32.) Æstivo-autumnal parasites. One rests on the periphery of the corpuscle with its pseudopodium overlying the red corpuscle in the form of a loop; the other is attached to a surface mound.

FIG. 240. (Corresponds to Fig. 38.) A tertian parasite resting on the periphery of a corpuscle with pseudopodia overlying the surface of the corpuscle.

FIG. 241. (Corresponds to Fig. 39.) A tertian parasite resting on the periphery of a corpuscle with pseudopodia overlying the surface of the corpuscle.

FIG. 242. (Corresponds to Fig. 42.) A tertian parasite resting on the periphery of a corpuscle, attached to a peripheral corpuscular mound.

FIG. 243. (Corresponds to Fig. 43.) A tertian parasite resting on the periphery of a corpuscle with pseudopodia overlying the surface of the corpuscle.

FIG. 244. (Corresponds to Fig. 45.) A tertian parasite resting on the periphery of the corpuscle with its pseudopodium overlying the surface of the infected corpuscle.

FIG. 245. (Corresponds to Fig. 61.) A pigmented tertian parasite resting on the periphery of a red corpuscle with pseudopodia overlying the infected corpuscle.

FIG. 246. (Corresponds to Fig. 62.) A pigmented tertian parasite partly pulled off a red corpuscle.

FIG. 247. (Corresponds to Fig. 57.) A pigmented tertian parasite attaching itself to a fresh red corpuscle.

FIG. 248. (Corresponds to Fig. 54.) A pigmented tertian parasite attaching itself to a fresh red corpuscle.

FIG. 249. (Corresponds to Fig. 52.) A pigmented tertian parasite partly pulled off a red corpuscle showing degenerative changes caused by the parasite.

FIG. 250. (Corresponds to Fig. 63.) A pigmented tertian parasite with its pseudopodium extending beyond the periphery of the infected corpuscle.

FIG. 251. (Corresponds to Fig. 138.) Æstivo-autumnal parasites with chromatin distorted by technique.

FIG. 252. (Corresponds to Fig. 123.) Æstivo-autumnal parasites. A free parasite may be seen at o. Distorted parasites are attached to the corpuscle.

FIG. 253. (Corresponds to Fig. 51.) A pigmented tertian parasite partly pulled off the infected corpuscle. The peripheral mound to which the parasite is attached may be seen at o. The corpuscle shows slight degenerative changes owing to the presence of the parasite.

FIG. 254. (Corresponds to Fig. 48.) A pigmented parasite partly pulled off the infected red corpuscle.

FIG. 255. (Corresponds to Fig. 130.) Distorted æstivo-autumnal parasites.

FIG. 256. (Corresponds to Fig. 139.) Distorted æstivo-autumnal parasites.

FIG. 257. (Corresponds to Fig. 144.) Distorted æstivo-autumnal parasites.

FIG. 258. (Corresponds to Fig. 132.) Distorted æstivo-autumnal parasites.

FIG. 259. (Corresponds to Fig. 137.) Distorted æstivo-autumnal parasites.

FIG. 260. (Corresponds to Fig. 145.) Distorted æstivo-autumnal parasites.

FIG. 261. (Corresponds to Fig. 141.) Distorted æstivo-autumnal parasites.

FIG. 262. (Corresponds to Fig. 142.) Distorted æstivo-autumnal parasties.

FIG. 263. (Corresponds to Fig. 133.) Distorted æstivo-autumnal parasites.

FIG. 264. (Corresponds to Fig. 87.) An æstivo-autumnal parasite with the chromatin of the nucleus drawn out in a long thread-like process far beyond the periphery of the infected corpuscle. This illustrates the large amount of chromatin in the nuclei of young parasites.

FIG. 265. (Corresponds to Fig. 75.) A free distorted tertian ring-form parasite.

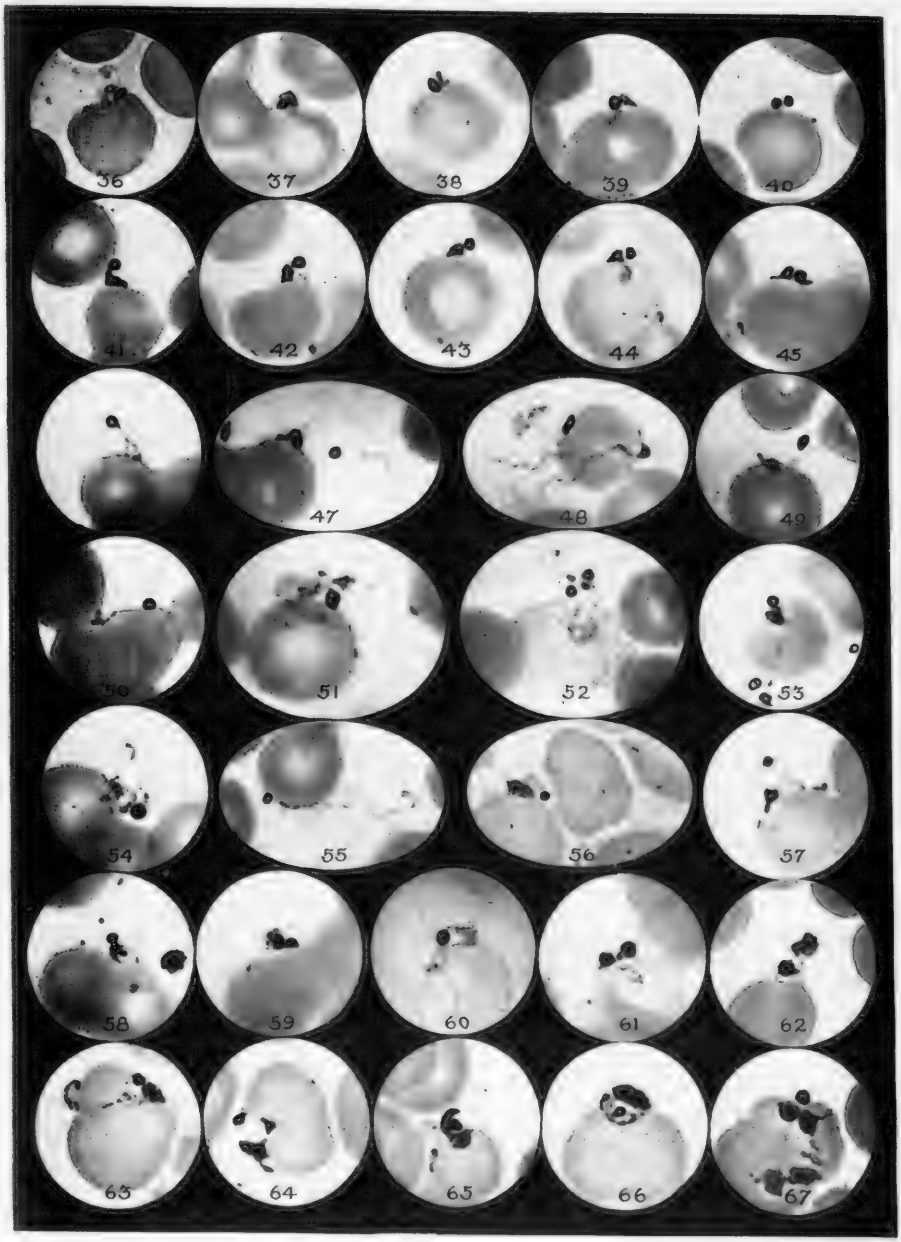
FIG. 266. (Corresponds to Fig. 107.) Distorted chromatin of æstivo-autumnal parasites whose cytoplasm has faded.

FIG. 267. (Corresponds to Fig. 116.) Distorted chromatin of æstivo-autumnal parasites whose cytoplasm has faded.

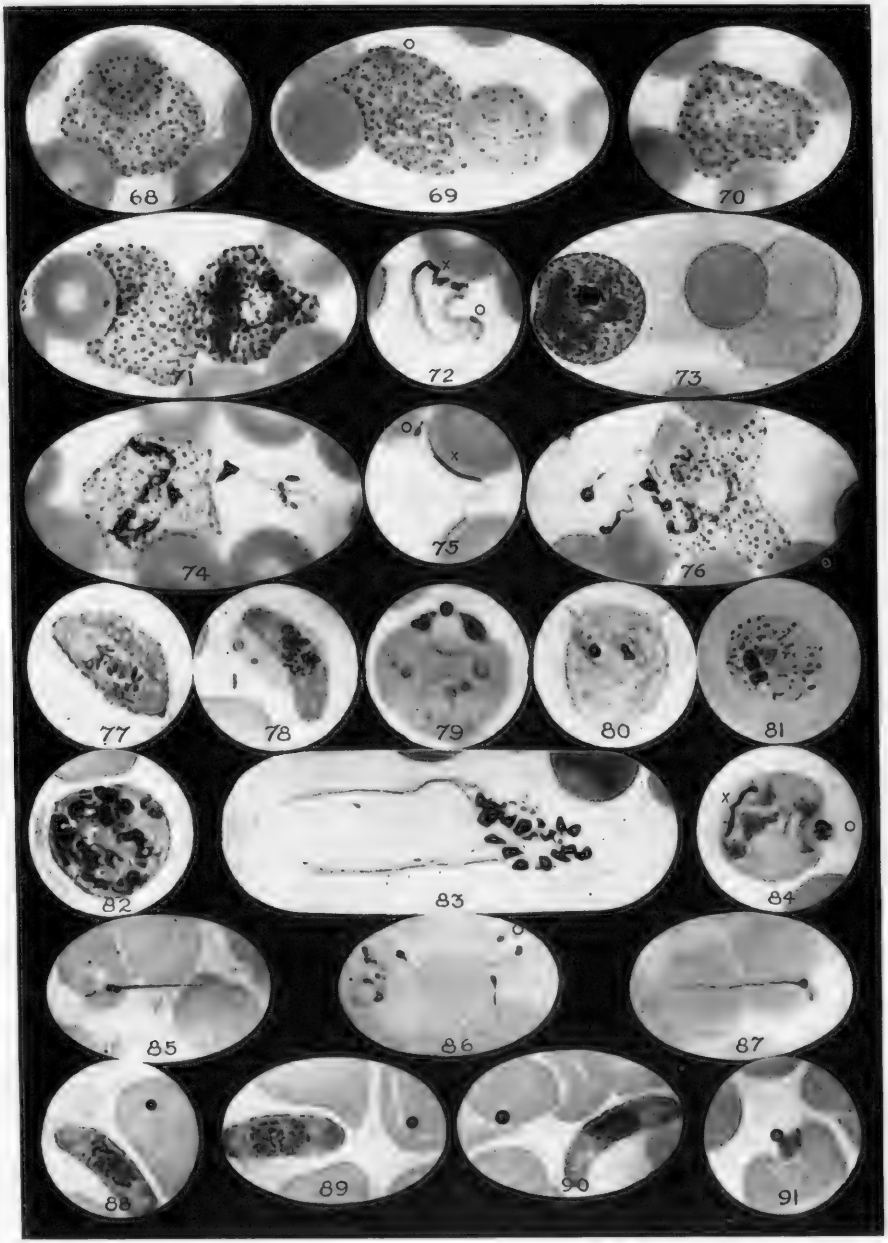
FIG. 268. (Corresponds to Fig. 103.) Cytoplasm of æstivo-autumnal parasites whose chromatin has faded.

FIG. 269. (Corresponds to Fig. 114.) Chromatin dots (nuclei) of æstivo-autumnal parasites whose cytoplasm has faded. These nuclei are normal in appearance.

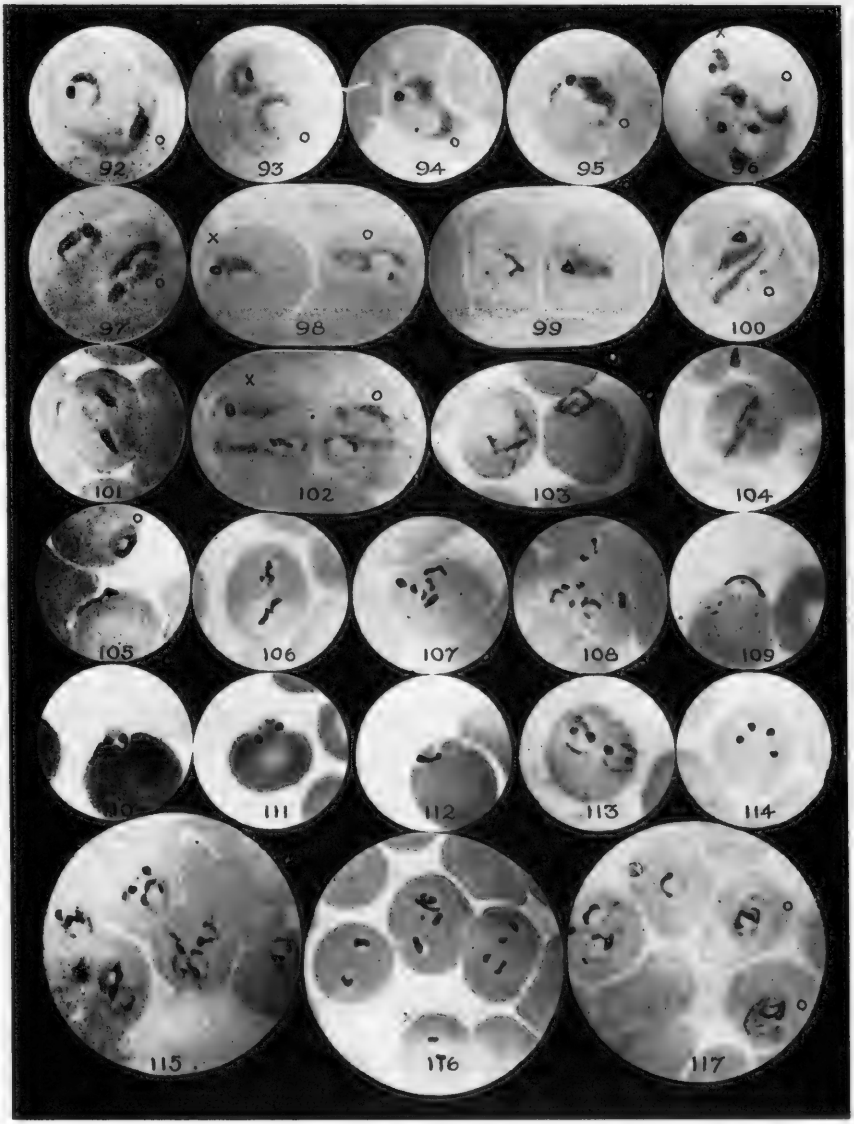
(Lawson: Distortion of the Malarial Parasite.)



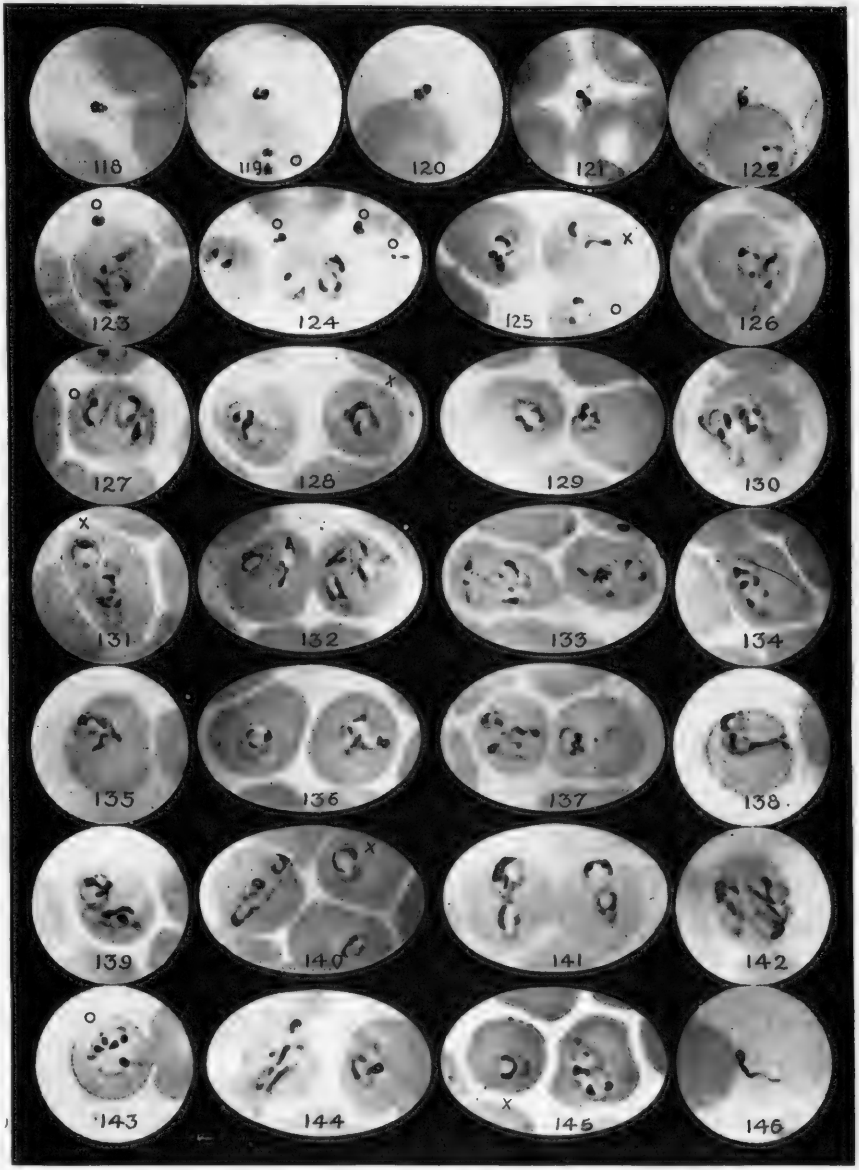
(Lawson: Distortion of the Malarial Parasite.)



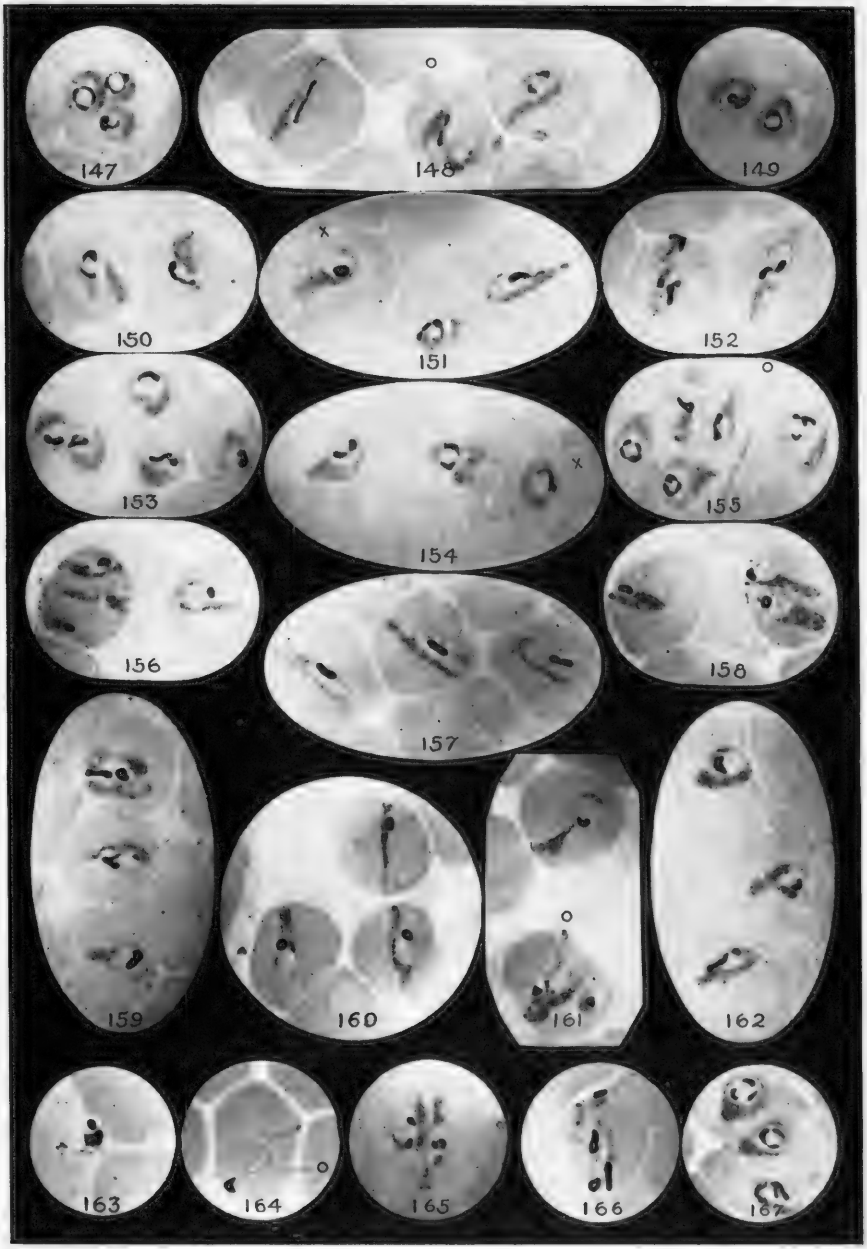
(Lawson: Distortion of the Malarial Parasite.)



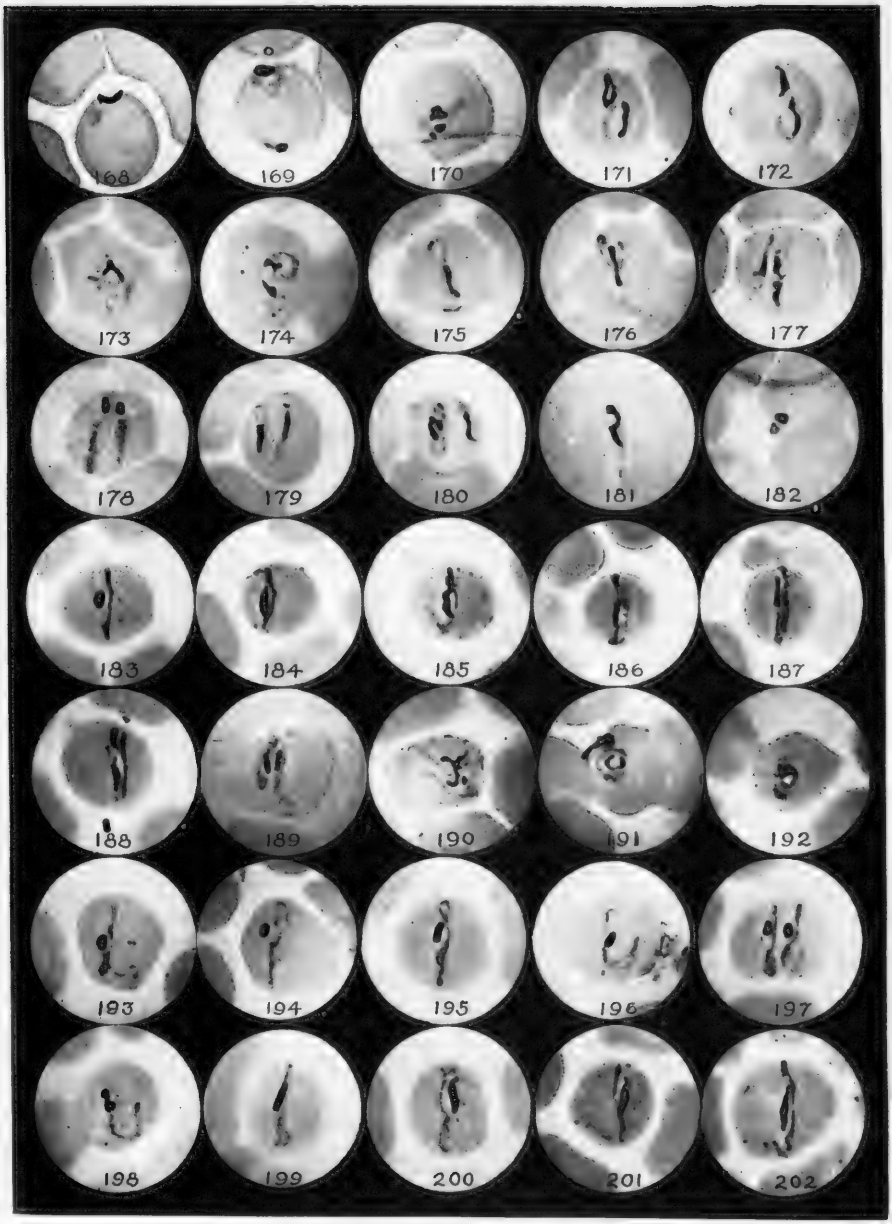
(Lawson: Distortion of the Malarial Parasite.)



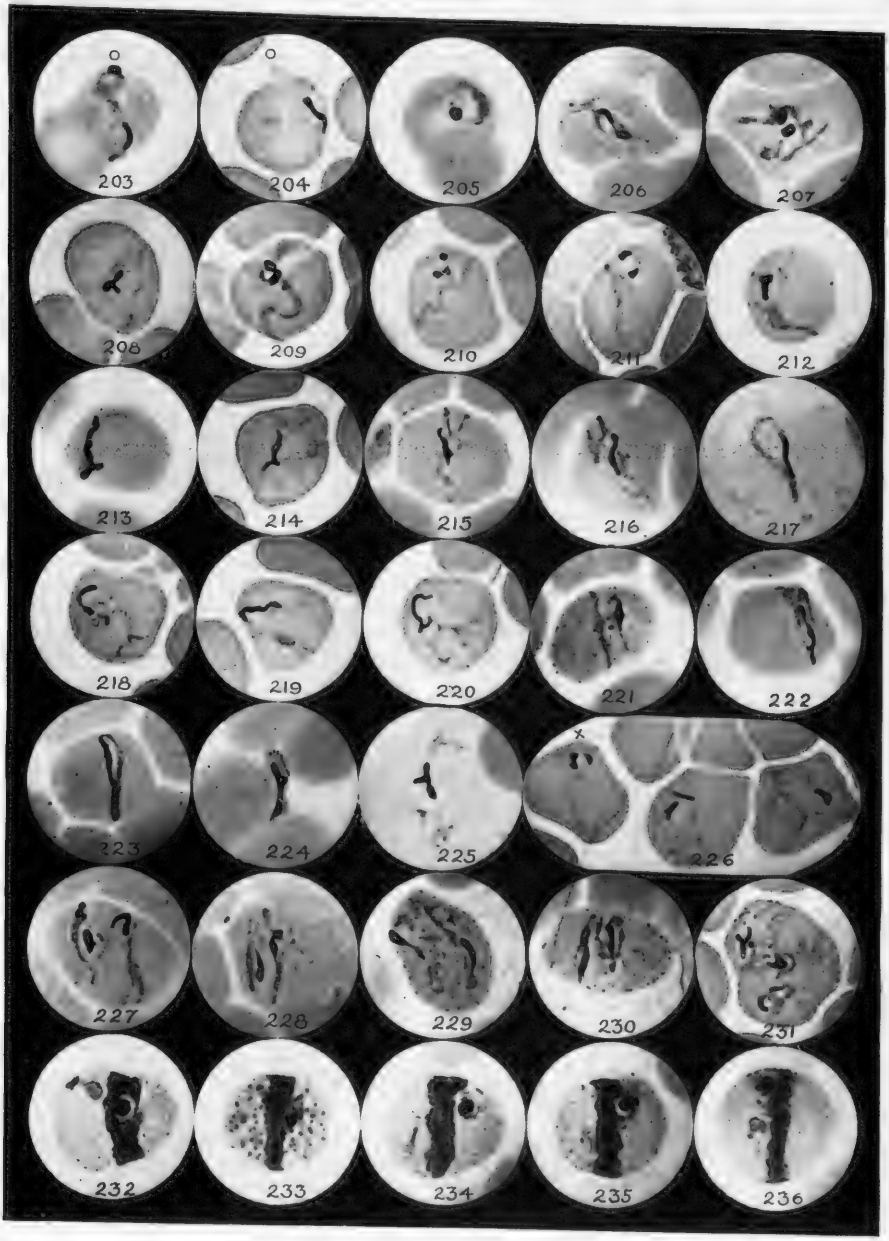
(Lawson: Distortion of the Malarial Parasite.)



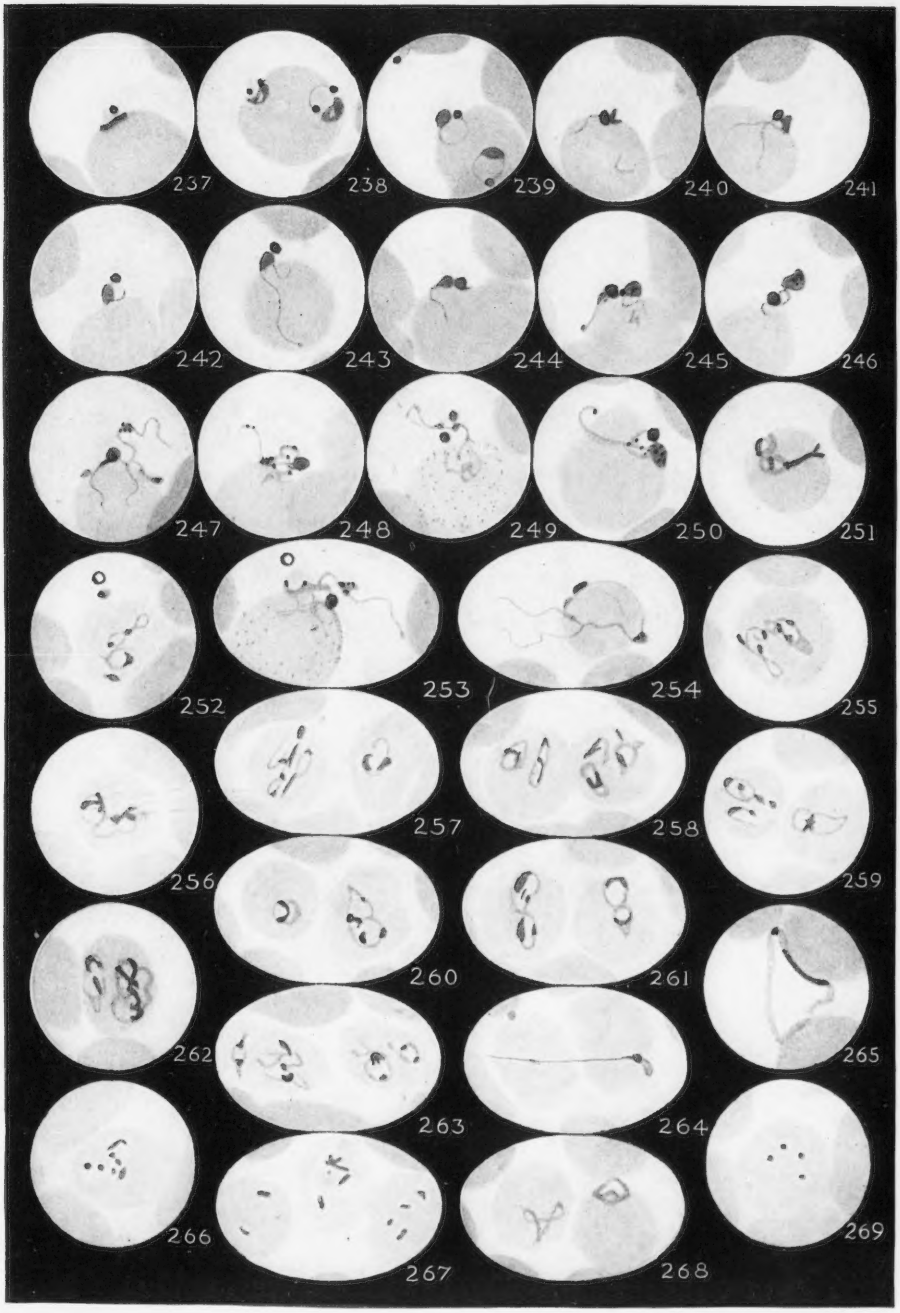
(Lawson: Distortion of the Malarial Parasite.)



(Lawson: Distortion of the Malarial Parasite.)



(Lawson: Distortion of the Malarial Parasite.)



(Lawson: Distortion of the Malarial Parasite.)